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QUANTITATIVE PHARMACEUTICAL CHEMISTRY

Quantitative Pharmaceutical Chemistry

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Fourth Edition 1953

McGraw-Hill Book Company, Inc.
NEW YORK TORONTO LONDON

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QUANTITATIVE PHARMACEUTICAL CHEMISTRY

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Library of Congress Catalog Card Number: 53-5558

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THE MAPLE PRESS COMPANY, YORK, PA.

PREFACE

trend in medical science makes pharmacy an increasingly
ant health profession. Large numbers of new drug products
een introduced and the quality control of these products has
ed greater importance in industrial and governmental lab-
ies. Progress has been so rapid that frequent editions of a
ok in the area of quantitative pharmaceutical analysis become
ary. The fact that previous editions of "Quantitative Phar-
tical Chemistry" have been received favorably as a valuable
nce by analysts and as an effective textbook by teachers and
nts of pharmacy makes this new fourth edition possible.

e the publication of the third edition of this book, numerous
es have been made in the official methods of analysis of phar-
tical materials in the United States Pharmacopoeia XIV and
National Formulary IX. The detailed procedures and dis-
ns have been changed or rewritten in the present edition to
rm to the revised official methods.

object of this book is threefold: First, to furnish students of
acy with a systematic course covering the quantitative chemi-
d physical methods official in the United States Pharmaco-
and the National Formulary through the selection and expla-
of typical procedures; second, to classify the large number of
l procedures according to type methods of analysis for effec-
nd convenient instruction; and third, to present some of the
ally applicable, nonofficial methods of analysis that are widely
in pharmacy and with which all students pursuing the profes-
hould be familiar. In seeking to attain all three objectives,
eory and practice of analytical chemistry as applied in quan-
ve pharmaceutical procedures have been correlated. Second-
jectives have been to adhere closely to unity of subject matter,
ce emphasis on the accuracy and proper technique required in
analytical and pharmaceutical practice, to present type meth-

ods in an orderly sequence leading from relatively simple to complex procedures, and to avoid expansion of the book to unwieldy size by inclusion of detail of subject matter that is readily available in general references.

The book is intended for use in didactic instruction and also as a laboratory manual. Its use as a text should be supplemented by lecture, recitation, and laboratory instruction. It is obviously impracticable to include detailed explanations of all the quantitative determinations in the United States Pharmacopoeia and the National Formulary. Typical analyses illustrating all the various methods have therefore been selected and explained in considerable detail, whereas those determinations requiring the same or closely similar procedures are indicated in tables or otherwise. The instructor may select other determinations from the Pharmacopoeia or the National Formulary and assign to the students the task of applying the theory and explanation of the procedure involved as given in the text.

The content of the book is arranged into three parts. Part 1 treats of general methods of gravimetric and volumetric analysis, Part 2 contains the special methods of pharmaceutical analysis, and Part 3 treats of physicochemical methods. Part 1 includes suitable material for a one-semester course. Parts 2 and 3 may be combined to constitute the work of the second semester.

The 28 chapters of the book have been thoroughly revised. The theory, 126 type procedures, and 75 tables have been rewritten in accord with the official requirements. New and additional questions and problems have been added to permit greater selection for assignment. New illustrations and much new material pertinent to instrumental methods have been introduced. The useful references to other textbooks and treatises on general and special methods of analytical procedure have been revised and extended.

The authors wish to acknowledge their appreciation of the courtesy extended by the following companies which granted permission to use illustrations from their catalogues and books: The American Pharmaceutical Association; The United States Pharmacopoeial Convention; The McGraw-Hill Book Company, Incorporated; Arthur S. LaPine and Company; The National Technical Laboratories; A. H. Thomas Company; Bausch and Lomb Optical Company; Central Scientific Company; Christian Becker, Incorporated; E. H. Sargent and Company; and Leeds & Northrup Company.

The wide popularity that the first, second, and third editions of this book have enjoyed among drug analysts, students, and teachers has been gratifying. It is hoped that the new edition will similarly commend itself to a still larger circle. To the many kind friends who have so generously aided by means of helpful suggestions, the authors desire to express their thanks.

The Authors

February, 1953.

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INTRODUCTION

Quantitative pharmaceutical analysis may be defined as the application of the procedures of quantitative analytical chemistry to the analysis of the materials used in pharmacy and, especially, to the determination of the purity and quality of the drugs and chemicals official in the United States Pharmacopoeia and in the National Formulary. A complete chemical analysis of any substance requires the establishment of the identity of its component parts by qualitative analysis and the determination of the proportion in which these components are present by the processes of quantitative analysis.

Quantitative chemical methods are commonly classified as gravimetric, volumetric, gasometric, and electrometric. It is impractical, however, to adhere strictly to this classification in presenting the subject matter of quantitative pharmaceutical analysis since certain phases of the work, as alkaloidal assaying and the assay of essential oils, have been developed to a point where they constitute a distinct subject matter in which the principles involved and the technique required are the same whether the procedure be gravimetric or volumetric.

Quantitative pharmaceutical analysis may be appropriately subdivided according to various procedures, each of which requires a special technique, as follows:

Gravimetric analysis, or the separation, by extraction, precipitation, or other means of the constituent to be determined either in the natural state, or in the form of a definite compound the composition of which is known to the analyst, and weighing the resulting product.

Volumetric analysis, or the determination of the volume of a solution of known concentration required to react with a given amount of the substance to be analyzed.

Gasometric analysis, or the measurement of the volume of a liberated gas or the decrease in volume of a mixture of gases when a suitable reagent is used to remove one of the gases present.

Special methods, or those which require a distinct type of technique, as alkaloidal assaying, require separate treatment in order to preserve unity of subject matter.

Physicochemical methods of analysis, or those which are based on the utilization of some specific physical or chemical property or properties of the substance in its quantitative estimation. Those physicochemical methods commonly applied in quantitative pharmaceutical chemistry are *optometric*, *refractometric*, *electrometric*, and *colorimetric*. They comprise some of the most valuable methods used in analytical procedure. Since each of them requires special treatment, they will be discussed separately.

The official assay methods serve as an exact measure of the purity of a substance only when the results are considered in conjunction with the qualitative tests. Thus, in the assay of zinc oxide, the purity of the oxide as determined by assay must follow qualitative tests for other metals which if present would be estimated as zinc oxide. A complete analysis, therefore, requires the qualitative estimation.

The theoretical considerations applicable in any given analysis are inherently a part of the analytical procedure. They should be mastered before a determination is made and carefully applied throughout each step of the procedure.

Those who desire a more comprehensive treatment of special aspects of analytical procedure will find the following texts and reference books of value:

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PART 1

GENERAL METHODS USED IN OFFICIAL PHARMACEUTICAL ANALYSES

The theoretical considerations and the procedures involved in gravimetric, volumetric, and gasometric methods of analysis are the same in quantitative pharmaceutical analysis as in quantitative chemical analysis. In quantitative pharmaceutical analysis, however, the general theories of analytical chemistry are applied to the procedures used in the analysis of the drugs, chemicals, and medicinal preparations employed in pharmacy; chiefly to those materials official in the United States Pharmacopoeia (U.S.P.) and the National Formulary (N.F.).

Chapter 1

REMARKS AND GENERAL DIRECTIONS

Success as an Analyst. To be successful as an analyst, the student must realize that analytical chemistry is not simple routine procedure. Manipulative skill acquired by experience with the ability to follow directions under the supervision of a skilled analyst may enable one to carry out successfully certain analytical procedures. An important objective in studying quantitative pharmaceutical chemistry, however, is the acquisition of a fundamental knowledge of the theory as well as a practical ability in application of analytical methods.

The successful analyst must know what reactions are taking place during an analysis and be able to understand and apply the theory upon which the method is dependent. The analyst must acquire skill of technique, patience, neatness, and accuracy. The fact should be borne in mind that analysis is carried out not for the sake of analysis but as a means of controlling the purity and strength of substances used as medicaments. Above all, to be successful, the analyst must not only use common sense in the laboratory but think throughout each step of the procedure.

Accuracy and Honesty. It is absolutely essential that all quantitative work be performed without even a slight loss of material or gain of extraneous matter. Every precaution should be taken to prevent such loss or gain. All solutions and precipitates should be covered to protect them from dust, whenever possible, and every particle of sample, solution, or precipitate must be regarded as indispensable to the ultimate success of the analysis.

Absolute integrity is demanded of every quantitative analyst. The operator is the only person who is familiar with the entire history of an analysis and who knows whether it is worthy of full confidence. When confidence is lost for any definitely established reason in the course of an analysis, operations should be discontinued at once, and a new start made. At least two determinations of any analytical value are required, and the results should agree closely. Failure to obtain duplicate values which check is conclusive evidence that at least one value is wrong and that neither value is dependable.

Notebooks. All notebooks should be of the type designated by the instructor. A small bound notebook which will slide under the balance while weighing has proved most satisfactory in the writer's experience.

The following information relative to each determination should be included:

Date.
Object or title.
Experimental data.
Reactions.
Calculations.
Results.
Remarks.

This information should be arranged in a neat, systematic manner so that anyone familiar with quantitative analysis may understand it readily.

The date is very important in all scientific work. Numerous legal decisions have been lost through the failure of the analyst to affix the date at the time of analysis. The object or title should be briefly stated and should identify the sample and the analytical value measured. The experimental data should include a complete record of *all* weighings and measurements set down at the time they are made. The practice of recording data on loose pieces of paper or in a scrap notebook and copying the original entries into a second notebook should not be tolerated. These data may be conveniently placed and followed by the calculations on the left-hand page of the notebook, leaving the right-hand page for a concise, orderly write-up of the entire experiment. All reactions should be given using structural formulas where possible. The use of logarithms for all calculations is highly recommended for accuracy and economy of time. Results should always be reported as percentages unless otherwise directed. Remarks should include any unexpected development during the analysis, explanation of error, and comments which seem pertinent to the technique or theory involved in the analytical method.

Economy of Time. Economical utilization of laboratory hours is best achieved through a preliminary study of the work to be done, followed by a plan for its prompt execution. There is no such thing as "speed" in quantitative work. Economy of time is achieved not by haste, but by planning the work so that two or more operations may be carried out at one time and especially by working so accurately that it will be unnecessary to repeat a determination. The following suggestions will save time and increase accuracy: (1) Keep the desk scrupulously clean at all times. (2) Clean all apparatus immediately after using it and put it in the desk. (3) Label all solutions, filtrates, and precipitates systematically throughout the analytical procedure. (4) Keep two or more operations going at one time; thus, while cooling a precipitate in the desiccator, make a weighing, or while washing one precipitate, ignite another. (5) Utilize all time between operations in making calculations and writing up experiments.

Cleaning Solution. A good cleaning solution can be prepared by trituration of 30 Gm. of commercial sodium dichromate or potassium dichromate with sufficient water to make a thick paste, and addition of 450 cc. of commercial concentrated sulfuric acid to the paste while stirring in a beaker.

The solution should be stored in a thick-walled, glass-stoppered, 500-cc. bottle and used repeatedly for cleaning glass and porcelain ware until it deposits a large amount of solid material or turns green.

Wash Bottles. Wash bottles for use in quantitative work usually consist of rubber-stoppered, flat-bottomed flasks of 500- to 1,000-cc. capacity, fitted with smoothly bent tubes and a jet made flexible by means of a rubber joint. The jet should be drawn out so that it will deliver a thin, even stream of liquid. Prepare two wash bottles of the type illustrated in Fig. 1, one of 1,000-cc. and one of 500-cc. capacity, for cold and hot water. The necks of wash bottles for use with hot water should be wrapped with asbestos paper or twine or other suitable material.

To bore a hole through a rubber stopper, dip the end of the borer in a strong solution of sodium hydroxide or in glycerin before starting to bore. Boil all rubber parts in dilute sodium hydroxide for from 2 to 3 min. and wash them thoroughly with distilled water. Fire polish the ends of all glass tubing by holding them in the blue flame of a burner until the sharp edges have become rounded.

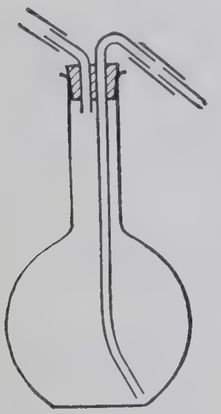


FIG. 1. A wash bottle for distilled water.

"Policeman." The so-called "policeman" is a piece of soft rubber tubing fitted to the end of a glass rod. The rod should be about 20 cm. long with a diameter between 5 and 7 mm., and it should have plain annealed ends. A piece of rubber tubing about 3 cm. long should be fitted tightly over, and project about 2 cm. beyond, the rod. The policeman is used to loosen from the walls of the containing vessel adhering particles that are not removable by a stream of water from the wash bottle. A policeman should never be used as a stirring rod or allowed to remain in analytical solutions.

Reagents. The reagents used in quantitative analysis must be pure. Reagents which conform to the requirements of the United States Pharmacopoeia and of the National Formulary should be used in all assays which have for their purpose the establishment of the strength or quality of official substances. Those reagents which meet the specifications of the American Chemical Society as well as those sold by reputable manufacturers as "analyzed" or "reagent" grade will usually be found to meet all official requirements.

Purity and Strength Requirements. The purity and strength of chemicals and drugs of the U.S.P. and of the N.F. are usually expressed in terms of *per cent.* The official requirements of purity and strength pertain only when the official methods of assay are employed. When the per cent strength or purity is given in round numbers, it is understood that the figures represent exactly the minimum or maximum requirement, *e.g.*, the requirement that hydrochloric acid contain "not less than 35 per cent and not more than 38 per cent of HCl" permits a product of 35 or 38 per cent or any intermediate strength; in other words, the figures are inclusive. It is established by rule that the figures 35 and 38, respectively, have the same significance as the figures 35.00 and 38.00.

Materials Required. The statement of materials required in the exercises is intended as a guide for the instructor in ordering supplies. Commonly used reagents and solutions, such as indicators, acids, and alkalies, are not always listed since it is assumed that they are available in the analytical laboratory. The quantities indicated are for each sample. It is possible to give only approximate quantities for materials used as solvents, for washing, for acidifying, and so forth. All reagent materials should meet the official requirements of purity and strength.

SAMPLING

The first important consideration in an analysis is the securing of a representative sample, a consideration too frequently ignored by analysts. Fixed rules cannot be laid down in sampling, for much depends on the nature of the material and the quantity from which a representative sample is to be taken. Whenever possible, a sample from each package should be examined separately. When a single package is sampled, the contents should be mixed or samples should be taken from different parts of the container and mixed, and then a portion of this mixture should be taken for analysis. The sampling of bulk quantities of chemicals and drugs may be performed by the methods given in the U.S.P. for sampling vegetable drugs:

a. It is recommended that gross samples of vegetable or animal drugs in which the component parts are 1 cm. or less in any dimension, and all powdered or ground drugs, be taken by means of a sampler which removes a core from the top to the bottom of the container, not less than two cores being taken in opposite directions; that when the total weight of the drug to be sampled is less than 100 kilos (200 pounds) at least 250 Gm. shall constitute an *official sample.* When the total weight of the drug to be sampled is in excess of 100 kilos, repeated samples shall be taken by the above method, and according to the schedule given below, mixed and quartered, two of the diagonal quarters being rejected, the remaining two quarters being combined and carefully mixed, and again subjected to a quartering process in the same manner until two of the quarters weigh at least 250 Gm., which latter quarters shall constitute an *official sample.*

b. It is recommended that gross samples of vegetable drugs in which the component parts are over 1 cm. in any dimension be taken by hand. When the total weight of the drug to be sampled is less than 100 kilos, at least 500 Gm. shall constitute an *official sample,* and this shall be taken from different parts of the container or containers. When the total weight of the drug to be sampled is in excess of 100 kilos, repeated samples shall be taken by the above method and according to the schedule below, mixed and quartered, two of the diagonal quarters being rejected, and the remaining two quarters being combined and carefully mixed, and again subjected to a quartering process in the same manner until two of the quarters weigh not less than 500 Gm., which latter quarters shall constitute an *official sample.*

SCHEDULE RECOMMENDED FOR SAMPLING

Number of Packages in Shipment	Number of Packages to Be Sampled
1 to 10	1 to 3
10 to 25	3 to 4
25 to 50	4 to 6
50 to 75	6 to 8
75 to 100	8 to 10

When over 100, the total number sampled should not be less than 10.

c. When the total weight of a drug to be sampled is less than 10 kilos, it is recommended that the above methods be followed but that somewhat smaller quantities be withdrawn, and in no case should the final *official sample* weigh less than 125 Gm.

d. In addition to the withdrawing of *official samples* according to methods *a*, *b*, and *c*, the *official sample* may consist of the total amount of a direct purchase made by Federal, State, or Municipal Food and Drugs Act enforcement officials.

Students' samples are supplied ready for analysis as a rule. Samples which require drying should be spread on a large watch glass, thoroughly mixed, and dried in an oven at the specified temperature. They should then be transferred to a weighing bottle and kept in the desiccator.

CALCULATION OF RESULTS AND ERRORS

Source and Nature of Errors. It is only in rare cases that the numerical value of an experimental result can be directly determined. As a rule, it is necessary to calculate the result from the different measurements and observations that have been made. Duplicate results that check very closely are not an assurance of accuracy, as is often taken for granted by the beginner in quantitative work, since errors inherent in the method and apparatus used may be repeated for each sample. Generally, results that agree closely when obtained by two different methods of analysis are a good indication of the reliability of the methods.

The results of repeated analyses or measurements will fail to agree, in general, when made by the same or different analysts to the full precision of which the method or instrument is capable. The discrepancies in the results are caused by various sources of error to which all experimental data are subject. The errors are of two types, *viz.*, indeterminate or accidental errors, which manifest themselves by slight variations in a series of observations made by the same observer under identical conditions. They result from causes difficult to detect, such as differences in the judgment and skill of the analyst. Generally, indeterminate errors are intangible, and their elimination by the analyst is impossible. Determinate or constant errors are of such nature that they recur in a constant manner in each of a series of determinations. Consequently, it is possible partially to determine their value and reduce their effect on the final result. They arise from causes such as (1) personal errors made by the individual analyst, *e.g.*, inability to judge color changes sharply, resulting in habitual reading of end points in titration too late; (2) errors of method caused by faulty procedure, *e.g.*, incorrect sampling, contamination of precipitates, and improper selection of indicators; (3) apparatus errors due to poor construction or calibration, *e.g.*, inaccuracy in the calibration of burettes or pipettes, inequality in the length of the arms of the balance, and incorrect weights. Errors of this type are usually detectable and so may be eliminated to a large extent.

Indeterminate Errors. The random distribution of indeterminate errors of various magnitudes is such that a graph showing the relationship between the magnitude of an error (abscissa) and the frequency of its occurrence (ordinate) has the shape of a normal frequency distribution curve or probability curve, sometimes called the *curve of error.*

An examination of this curve shows that (1) very large errors are unlikely to occur often; (2) small errors occur with greater frequency than large ones; and (3) positive and negative errors of the same numerical magnitude are equally probable since the curve is symmetrical with respect to the Y axis. Consequently, it is apparent that the best value to select for a series of observations is their arithmetical mean.

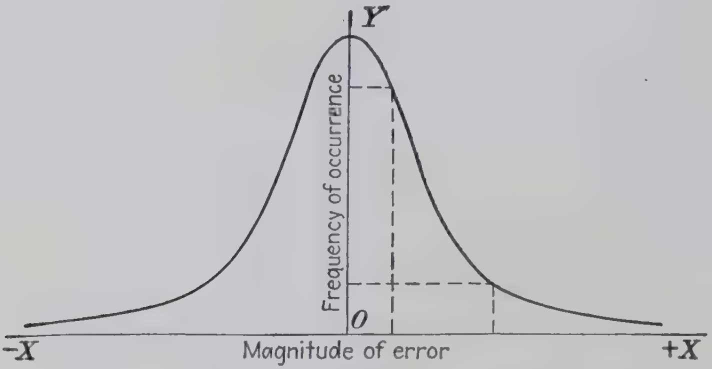


FIG. 2. Probability curve.

Average Deviation from the Arithmetical Mean. In order to measure the magnitude of indeterminate or accidental errors, use is made of the theorem proved by the method of least squares, which states that an arithmetical mean computed from n equally trustworthy observations is $\sqrt{n}$ times as trustworthy as any single observation. If the indeterminate error in any one observation is represented by d, the indeterminate error of the arithmetical mean is $d/\sqrt{n}$; in other words, the uncertainty of the arithmetical mean is inversely proportional to the square root of the number of observations. The deviation of a single measurement from the arithmetical mean of a series of similar measurements can readily be determined and from this the average deviation of a single determination and the average deviation of the mean value can be calculated, as in the following example.

In the standardization of hydrochloric acid by titration of samples of pure sodium carbonate, the burette readings 30.25, 30.22, 30.26, 30.27, and 30.23 cc. were obtained. The arithmetical mean of these numbers is 30.246. The deviations of the individual readings from the mean, neglecting sign, are 0.004, 0.026, 0.014, 0.024, and 0.016.

The sum of these five numbers is 0.084, and the average deviation of any of the individual values from the mean value is

$$\frac{0.084}{5} = 0.017 \qquad \text{or} \qquad \frac{0.017}{30.246} \times 1{,}000 = 0.56 \text{ part per thousand}$$

The average deviation of the mean value, however, is considerably less and is equal to

$$\frac{0.017}{\sqrt{5}} = 0.008 \qquad \text{or} \qquad \frac{0.008}{30.246} \times 1{,}000 = 0.26 \text{ part per thousand}$$

From any one of the individual values and the average deviation of an individual value from the mean, a range of values which probably includes the true value can be calculated; *e.g.*,

$$30.25 \pm 0.017 = 30.23 \text{ to } 30.27$$

These values have been rounded off to the nearest 0.01 cc. Because of the greater precision of the mean value, the range of values which probably includes the true value and which can be calculated from the mean and the average deviation of the mean, is much smaller:

$$30.246 \pm 0.008 = 30.24 \text{ to } 30.25$$

Sometimes individual determinations of a given value will agree closely because the indeterminate error is small, but the mean value calculated from them will not be a satisfactory estimate of the true value because determinate error is large. For example, a sample known to contain 60.05 per cent chlorine as chloride was assayed for chlorine and the following values were obtained: 57.63, 57.68, 57.66, and 57.63. The mean calculated from these values is 57.65, and the average deviation of the individual values is only 0.02 or 0.35 part per thousand. It is obvious from the close agreement between the individual values and the mean value that the indeterminate error of the analysis is very small, or the *precision* of the measurement is high. When the mean value obtained in the analysis is compared with the true value (60.05) and the absolute error calculated,

$$60.05 - 57.65 = 2.4 \qquad \text{or} \qquad \frac{2.4}{60.05} \times 1{,}000 = 40 \text{ parts per thousand}$$

a very large determinate error in the analytical procedure is apparent, and the *accuracy* of the determination is low.

Rejection of a Result. Sometimes in a series of determinations of an analytical value, one of the results obtained will differ greatly from the others although no mistake was known to have been made in the work. The question of retaining or rejecting the apparently aberrant result in computation of the arithmetic mean then arises. If at least four determinations have been made, the result in which the error appears to be large may be rejected when the deviation of the questionable value from the arithmetic mean of the other values is four times the average deviation of the other values from the mean. For example, in a series of determinations of the NaCl content of a sample, the following values (percentages) were obtained: 95.72, 95.81, 95.83, 95.92, and 96.18. The arithmetic mean of the first four values is 95.82 and the average deviation of the individual values from the mean is 0.055. The deviation of the last value (96.18) from the mean of the other four values (95.82) is 0.36 or $0.36/0.055 = >6.5$ times the average deviation of the other values from the mean. Consequently, the last value should be rejected since it has been shown that the deviation of a result which is four or more times the average deviation is due to a mistake in all except about five cases in a thousand.

Significant Figures. Significant figures are the digits that when placed in order give the value to a number, *e.g.*, in the quantity represented by the number 236, the digits 2, 3, and 6 are significant figures. Zeros are employed in some cases to locate the decimal point; in other cases, they may be significant figures. Thus in the number 20.5, the zero is a significant figure, but in the number 0.0125 the zeros are not significant but show the order of magnitude of the other digits. In the quantity 1.0000 Gm., the zeros are significant since they indicate that the weighing has been made to $\frac{1}{10}$ mg. and that only the last zero is uncertain.

Every measurement involves some error or can be carried out only with a certain degree of accuracy. Consequently, it is evident that the number expressing the value of a measurement can be only approximate. Irrespective of the number of figures written as the result of a calculation, the accuracy of a value beyond that limited by the errors of the measurements cannot be increased. On the other hand, if too few figures are written down, the resulting value may be much less accurate than the data permit. The proper manner of expressing a result is to retain or use such a number of figures that all, except the last, are known with certainty, and that although the last figure is uncertain the error is not greater than ±5 in the following place. For example: If a burette can be read to 0.01 cc. and 32.5 instead of 32.50 is written as the result of a measurement, the mistake of writing too few figures is committed since the number 32.5 indicates that the true value lies between 32.45 and 32.55 and the apparent error is five times greater than the error of measurement. Again if, in recording the result of a series of burette readings, the mean of the values 32.52, 32.54, 32.54, and 32.55 cc. is written as 32.538, it would be a mistake of retaining too many figures, since it would indicate that the error of measurement is only about ±0.0005 cc. The number should be rounded off, therefore, to 32.54 cc. For purposes of further calculation, all the figures should be retained; *i.e.*, the number 32.538 should be used.

In general, for analytical work, the operations of addition and subtraction should give as the final result no more decimal places than the least number of decimal places entering into the calculation. This is illustrated by the following example: In the addition $12.4 + 121.502 + 3.6653 = 137.5673$, the maximum apparent error in the result is ±0.05, which is the greatest apparent error in the number 12.4. Therefore, a derived error of ±5 exists in the second decimal place, figure 6, of the result, and the result should be written 137.6, but if the result is used for further calculations, it is better to retain one figure more, *i.e.*, to employ 137.57.

In multiplication or division, as a rule, retain one more significant figure, when available, in the result than there are in the number having the least number of significant figures. For example: The product of $11.32 \times 12.2 \times 0.0321 = 4.4331384$. If the last figure in each number is uncertain, the relative error in the number 12.2 will be about 5 in 1,200 or 0.4 per cent. Consequently, it would be incorrect to write the result as 4.4331384, for this result has a derived error of 0.4 per cent or of about 2 units in the second place of the decimals. All figures following this are therefore meaningless and should be discarded, the result being written 4.43.

Logarithms and Their Use. The solution of problems and the calculation of the results of analyses from data are greatly facilitated by the use of logarithms. It is advisable for students to make calculations by the use of logarithms in order to save time and avoid tedious calculations by longhand.

A logarithm is an exponent that must be applied to a fixed positive number other than 0 or 1 to produce any given number. The fixed number is called the base. In the common or Briggsian system of logarithms, the base is 10. A logarithm consists of two parts, the characteristic or integral part and the mantissa or fractional part. The characteristic of a number greater than 1 is always one less than the number of digits to the left of the decimal point, *i.e.*, where n equals the number of digits to the left of the decimal point, the characteristic is represented by a number equal to $n - 1$. For example, the characteristic of 10,000 or 10^4 is 4, of 100 or 10^2 is 2, and of 1 or 10^0 is 0. The characteristic may be either positive or negative. When there are no digits to the left of the decimal point, the characteristic is negative or minus, and when there are no zeros before the significant figures to the right of the decimal point, the characteristic is -1 or $9 - 10$. When there are no digits to the left of the decimal point and n zeros to the right of the decimal point before the significant figures, the characteristic is $(9 - n) - 10$, *e.g.*, the characteristic of 0.1 is $\overline{1}$ or $9 - 10$, of 0.01 is $\overline{2}$ or $8 - 10$, and of 0.000001 is $\overline{6}$ or $4 - 10$.

The mantissas of numbers are furnished by the table of logarithms, see page 509. The examples given below illustrate the use of the tables.

Example 1. Find the logarithm of 9,465. From the explanation given, it is evident that the characteristic is 3. To find the mantissa, locate 94 in the logarithm table in the column of Natural Numbers and then move directly to the right to the column under 6 at the top of the page. The mantissa given there is 9759. This is the mantissa for 9,460. To find the mantissa for 9,465, move farther to the right to the columns headed Proportional Parts where, under the 5 column, the figure 2 is given. This figure 0002 when added to 9759 shows how much more the logarithm of 9,465 is than that of 9,460. Consequently, the logarithm of 9,465 is

$$3.9759 + 0.0002 = 3.9761$$

Example 2. Find the logarithm of 0.0001058. Since the first significant digit is four places to the right of the decimal point, the characteristic is 6 − 10. Find the mantissa as in example 1 by following the horizontal line opposite 10 to the vertical column headed by 5 and add to this the proportional part under the column headed 8. The mantissa is then found to be 0212 + 0033 = 0245, and the logarithm is written 6.0245 − 10.

Example 3. Multiplying and dividing with logarithms.

To multiply, add the logarithms of the numbers together. To divide subtract one logarithm from the other. Study the following example:

$$\frac{24.46 \times 0.2970}{1{,}000 \times 0.003741}$$

Multiply by adding the logarithms

log 24.46 . . . . = 1.3885
log 0.2970 . . . . = 9.4728 − 10
log (24.46 × 0.2970) is the sum of the logs . . . . = 10.8613 − 10
log 1,000 . . . . = 3.0000
log 0.003741 . . . . = 7.5730 − 10
log (1,000 × 0.003741) is the sum of the logs . . . . = 10.5730 − 10

Divide by subtracting the logs

10.8613 − 10
10.5730 − 10
0.2883

The result of these operations is the logarithm of the number sought. To find the natural number corresponding to the logarithm 0.2883, turn to the table of antilogarithms (page 511), move down the column headed Logarithms until 28 is found, move horizontally to the column headed 8 where the number 1941 is found, and then proceed to the Proportional Parts column under 3 where the number 0001 is found; add this to 1941, the sum is 1942. The characteristic is 0. Therefore the one digit is to the left of the decimal point and the required number is 1.942.

Questions and Problems

1. Rewrite each of the following quantities, underscoring the significant figures: (*a*) 1.1200 Gm. weighed on the analytical balance; (*b*) 20.459 cc. from a burette reading; (*c*) 0.0062 Gm. from a weighing on the analytical balance.

2. Make the following calculations retaining only the significant figures:

a. $2.3 + 100.56 + 4.273 - 20.005 =$
b. $100 \times 0.010 \times 1{,}000 \div 10 =$
c. $(20 \times 1.0250) - (10 \times 1.2000) \div 1.200 =$

3. A sample known to be 98.50 per cent pure yielded results on analysis in duplicate of 98.30 and 98.12 per cent, respectively. What is the error in parts per thousand of each determination?

4. The result of an analysis for per cent purity employing the usual apparatus and weighing to the fourth decimal place was reported as 86.3584323 per cent. How should the result be reported?

5. The following results were obtained in the determination of chloride ion in a sample of pure potassium chloride: 35.26, 34.98, 35.00, and 35.07 per cent. Calculate: (*a*) the mean deviation; (*b*) the per cent deviation; (*c*) the mean deviation in parts per thousand; (*d*) the mean error in per cent.

Should any of the results be rejected? Why?

6. Find the common logarithms of the following numbers: 100, 65, 8,542, 0.221, 0.00018.

7. Find the numbers (antilogarithms) that correspond to each of the following logarithms: 3.5448, 0.8250, 0.1260, 2.3979, 1.6990, 8.4321–10.

8. Using logarithms, multiply: (*a*) $21{,}500 \times 0.000332$; (*b*) 0.0648×0.0008426; (*c*) $(42.16 - 1.85)(36.20 + 12.82)(0.5444 \times 0.1112)$.

9. Using logarithms, divide: (*a*) 0.01648 by 0.9472; (*b*) 20.04 by 140.80; (*c*) 1,020 by 12.64.

10. Look up the logarithm to five places of 2 and 3; it is advisable to fix these values firmly in your mind. Using these values, and without further reference to tables, calculate the logarithms of the following numbers:

4
5
6
8
9
10
12
15
16
18
20

1.0
1.2
1.333
1.5
1.6
1.8
2.0
2.22
2.4
2.5
2.7

111
120
133
144
150
160
180
192
288
333
480

25
27
30
32
36
40
45
50
54
60
64

$\frac{1}{4}$
$\frac{1}{3}$
$\frac{2}{3}$
$\frac{5}{18}$
$\frac{1}{8}$
$\frac{2}{15}$
$\frac{9}{32}$
$\frac{25}{64}$
$\frac{16}{81}$
$\frac{27}{125}$
$\frac{125}{25}$

0.9
0.09
0.009
1.0
0.1
0.01
0.001
0.000001
0.125
0.375
0.875

11. Using the slide rule, solve the following;

a. 27×32
b. 272×328
c. 275×327
d. 271×3.25
e. $0.279 \times 3{,}290$
f. 27.4×0.0326
g. 875×24.6
h. 0.933×1.926
i. 0.00453×0.000345
j. $777 \times 853 \times 269$
k. $12.41 \times 0.567 \times 9.05$
l. $45/9.3$
m. $4.5/9.3$
n. $0.45/9.3$
o. $^{875}/_{264}$
p. $^{125}/_{55}$
q. $0.01565/0.1655$
r. $\dfrac{377 \times 11.25}{75.3}$
s. $\dfrac{0.981 \times 181.9}{652}$
t. $\dfrac{0.0874 \times 0.247}{0.00875}$
u. $\dfrac{99.9 \times 88.8}{77.7 \times 66.6}$
v. $\dfrac{1{,}012 \times 0.001023}{0.0056 \times 0.1049}$
w. $\dfrac{1{,}003 \times 0.963}{803 \times 0.005}$
x. $\dfrac{\dfrac{6.05}{1.03} \times 2.07}{91 \times \dfrac{0.0001}{0.0009}}$
y. $\dfrac{283 \times 1.411 \times 964 \times 0.333 \times 50.2}{0.032 \times 385 \times 7.34 \times 90.0 \times .431}$

12. What is the approximate difference in parts per hundred between the following pairs of figures:

a. 100 101	*b.* 99 101	*c.* 200 201	*d.* 202 203	*e.* 4,001 4,006
f. 51 50	*g.* 51.0 51.5	*h.* 50 52	*i.* 25 24	*j.* 10 11
k. 155 154	*l.* 15.5 15.4	*m.* 1.55 1.56	*n.* 0.155 0.156	*o.* 0.0155 0.0154
p. 813 805	*q.* 19.8 20.1	*r.* 2.54 2.49	*s.* 0.0999 0.1004	*t.* 87,654 87,599
u. 0.000032 0.0000325	*v.* 0.000091 0.000092	*w.* 0.0056781 0.0056805	*x.* 1.00001 0.98032	*y.* 3,579,028 3,578,799

GENERAL OPERATIONS

Crucibles. A variety of crucibles are used in quantitative analysis. Those most commonly employed are made of high-grade porcelain. They withstand high temperatures and are suitable for use in the ignition of most drugs and precipitates. They are not suitable for fusions because the glaze is attacked by the flux, especially if the flux is basic. Other crucibles consist of fused silica, alundum, nickel, and platinum.

The Gooch Filtration Crucible. The Gooch filtration crucible (Fig. 3) is designed for the separation of precipitates by suction filtration. It has a perforated bottom upon which is bedded a mat of asbestos, thus making it possible to collect, wash, dry, and weigh a precipitate in the same crucible.

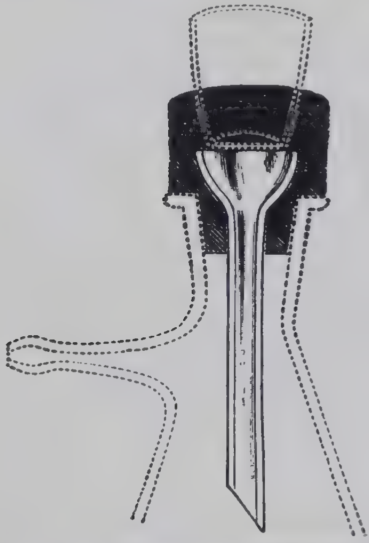


FIG. 3. Diagram of Gooch crucible assembly for filtration.

Preparation and Use of Gooch Crucible. Obtain about 0.5 Gm. of asbestos fiber (washed with hydrochloric acid) and shake it thoroughly with 100 cc. of distilled water in a suitable flask. Allow the asbestos to settle, and decant most of the water containing the fine fibers. Add about 100 cc. of water and agitate the mixture again. Fit the Gooch funnel containing the crucible in the mouth of a filter flask connected to a suction pump. Pour the suspended asbestos mixture into the crucible in small successive portions, applying suction gently after the addition of each portion until a smooth mat not over 1.5 mm. thick is obtained. Upon holding the crucible to a strong light, the holes in the bottom should be just perceptible and the mat of asbestos should appear uniform. Cover the asbestos with a perforated porcelain disk (filter plate) and add a very thin layer of asbestos. Wash the filter mat with 200 to 300 cc. of water or until no trace of asbestos fiber passes into the washing. Place the prepared crucible on a watch glass and dry it for 1 hr. in an oven at 120 to 130°. Allow the crucible to cool in a desiccator, then weigh. Repeat until the weight of the prepared crucible is constant within 0.0002 Gm. for two successive weighings.

To use the Gooch crucible, place it in the funnel and apply moderate suction. Pour the liquid from the precipitate into the crucible, wash the precipitate in the beaker by decantation, transfer the precipitate to the crucible, and complete the washing. The rod used to guide the liquid into the crucible should never be allowed to come in contact with the asbestos mat, since it may break the thin film above the filter plate. The filtrate should be examined for traces of asbestos fiber, and if such are found, it should be repassed through the filter.

A properly prepared Gooch filter may be used for a number of successive determinations where the precipitates are coarse-grained and crystalline.

Fritted-glass Crucibles. Fritted-glass crucibles (Fig. 4) have fused in sintered or fritted-glass bottoms and are supplied in various porosities such as "medium" and "fine." Crucibles of medium porosity are suitable for the filtration of flocculent and moderately fine precipitates. Crucibles of fine porosity are suitable for the filtration of fine precipitates such as mercuric or bismuth sulfides. These crucibles are useful in analyses where the residue is to be dried to constant weight at temperatures below 150°. The crucibles should be heated in an oven and allowed to cool to room temperature in a desiccator.

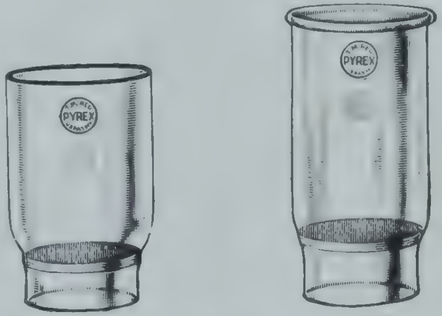


FIG. 4. Fritted glass crucibles, low form and high form.

Filtration and Washing Precipitates. The most usual and efficient method of washing a precipitate is by the process of decantation. The liquid above the precipitate is poured, using a stirring rod as a guide, onto a prepared filter, some of the liquid and most of the precipitate being allowed to remain in the container. A given amount of wash water is then poured on the precipitate and stirred vigorously. The precipitate is allowed to settle, and the supernatant liquid is decanted as before. This process is repeated three or four times. The precipitate is then washed onto the filter, using a "policeman" if necessary to dislodge any particles that adhere to the container wall. The filter containing the precipitate is washed by causing a fine stream of liquid from the wash bottle to fall upon the filter, always washing from the top edge down, until the filter is about three-fourths full. The filter is allowed to drain until practically empty, then the rinsing is repeated. After about five such rinsings, 1 or 2 cc. of the final filtrate should be tested for completeness of washing.

It should be borne in mind that it is better to wash a precipitate a comparatively large number of times with small portions of liquid than only a few times with large portions and that each portion of wash liquid should be removed as completely as practicable before the next portion is added. Never fill the filter more than to within $\frac{1}{4}$ in. of its upper edge. The size of the filter should be governed by the amount of precipitate, not by the amount of liquid to be filtered.

Colloidal and Fine-grained Precipitates. Colloidal precipitates, some of them gelatinous, some of them finely divided, are given by the most insoluble substances, such as sulfides, hydroxides, elementary sulfur, etc. Although insoluble, they have the property of being converted by water into colloidal dispersions, which pass through the filter. These substances can usually be coagulated and rendered filterable by boiling them after the addition of an electrolyte, such as NH_4NO_3, NH_4Cl, or HCl. It is in part for this reason that prepared wash liquors, rather than pure water, are used for washing certain of these precipitates.

All crystallization is preceded by supersaturation. If supersaturation is high, crystallization will be spontaneous and the crystals formed will be so fine that they will pass through the filter. If the precipitating agent is added slowly with constant stirring, high local supersaturation will be avoided, and the crystals formed will grow in size to such an extent that they will be retained by the filter. By using different concentrations, it is possible to get the same salt crystallized out in crystals varying in size from full-faced crystals down to the colloidal form.

Evaporation of Liquids. The evaporation of liquids is best carried out in porcelain dishes constructed for that purpose, which expose a large surface of liquid to the air. Evaporations should, with few exceptions, be carried out on the water bath to avoid danger of loss of material due to spattering or bumping and to avoid decomposition. The dish should be covered by an inverted funnel supported above it or with a watch glass elevated above the rim of the dish by means of a glass triangle to prevent the entry of foreign matter into the liquid. Large quantities of liquid are best evaporated in a comparatively small dish by adding fresh portions of the original liquid from time to time to replace that lost by evaporation. This helps to eliminate the source of error involved in the frequent transfer of liquids.

Transfer of Liquids. As few transfers of liquid as possible should be made, since each is a possible source of error. When transfers are necessary, they must be made quantitatively, washing the original container with successive small portions of wash liquid. This procedure is the most efficient, since it most thoroughly removes the material and keeps the volume of liquid within workable limits.

When transferring liquids from one vessel to another, a guide rod should always be used. It should be held tightly against the lip of the container.

Drying and Ignition of Samples and Precipitates. Many substances are directed to be dried under specified conditions before analysis to correct for absorbed moisture. Precipitates frequently must be dried previous to ignition. Numerous types of drying ovens are marketed that are suitable for this purpose, the electrically heated ones being best suited to constant temperature control. Samples should be dried at the specified temperature on a watch glass or in a suitable container. Precipitates may be dried directly on a filter by placing the funnel containing both directly in a drying oven adjusted to a temperature of 90 to 100° unless otherwise specified. The funnel should be covered with a sheet of filter paper held in place by crimping its edges over the rim of the funnel.

Precipitates may frequently be ignited without previous drying. To do this, the precipitate is folded within the filter paper, and the whole placed in a vertical crucible. The crucible is covered and heated gently by intermittent application of the flame until all the moisture is driven off and the paper is well charred. The cover is then removed, and the crucible placed in an inclined position on the triangle (Fig. 5). The full flame is then turned on and ignition continued until all carbon has been burned away, rotating the crucible slightly from time to time to expose fresh portions of precipitate. The crucible containing the ignited residue is allowed to cool in a desiccator and weighed. It is then ignited a second time and weighed. If the successive weighings do not agree within 0.2 mg. when a porcelain crucible is used, it should be ignited and weighed a third time. These operations should be repeated until constant weight is attained.

Constant Weight. The official methods often direct that a drug be dried to constant weight. The term *dried to constant weight* means that two consecutive weighings do not differ by more than 0.5 mg. per Gm. of substance taken for the determination, the second weighing following an additional hour of drying. The term *constant weight* is used more often in a different sense in analytical procedures. If the exact weight of an object, *e.g.*, a crucible, is desired, the object is heated at the specified temperature, cooled, and weighed. The object is reheated, cooled, and again weighed. If the two weights agree within 0.3 mg., constant weight is said to have been reached. If the change in weight is more than 0.3 mg, the process of heating, cooling, and weighing is repeated until con-

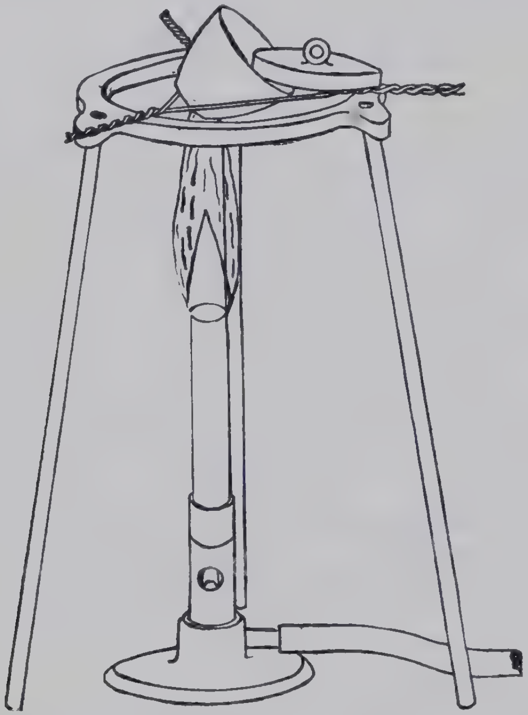


FIG. 5. Position of crucible above flame during ignition.

stant weight is obtained. An object is said to be at constant weight when two consecutive weighings after heating and cooling do not differ by more than 0.3 mg.

Use of Desiccators. Desiccators are a special form of glass vessel, rendered airtight by means of ground contact surfaces, used to maintain a dry atmosphere for objects that might be affected by moisture or carbon dioxide. If the contact surfaces are not airtight, they may be made so by coating them with a thin layer of grease prepared by melting together equal parts of beeswax and petrolatum. The desiccator is usually divided into two compartments separated by a perforated porcelain plate. The lower compartment is charged with a dehydrating agent, one of the most common of which is anhydrous calcium chloride, but concentrated sulfuric acid, solid potassium hydroxide, unslaked lime, magnesium perchlorate, etc., are sometimes used. The porcelain plate is usually fitted with holes for crucibles. The desiccator should always be kept closed when not transferring crucibles into or from it.

THE ANALYTICAL BALANCE

A knowledge of the principles and use of the analytical balance (Fig. 6) is essential in all quantitative chemistry. The diagram (Fig. 7) shows the various parts of such a balance with the proper name of each part. Other balances on the market differ slightly in construction, but the parts are essentially the same for all. The analytical balance is a delicate instrument of precision which must be handled with extreme care to maintain its fine adjustment and accuracy. Each student, under the direct supervision of an instructor, should learn the name and function of each part.

The following brief comments will serve to assist the student better to understand the function and care of the balance: The balance beam is supported on a center knife-edge or fulcrum, usually of agate, which rests upon an agate plate. Two pans intended to support the masses to be compared are suspended vertically from each end of the beam by stirrups. Each stirrup has an agate bearing which rests on a knife-edge. The arms of the balance beam are graduated so that a rider, of known weight, can be placed at any desired distance from the central knife-edge. Forcible or sudden

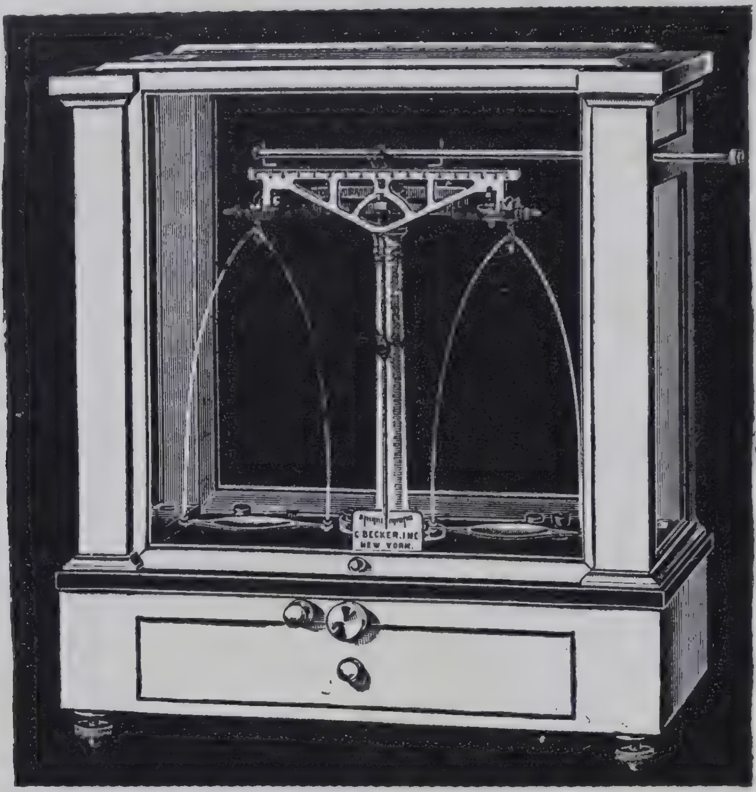

C. BECKER, INC.
NEW YORK

Fig. 6. A typical analytical balance.

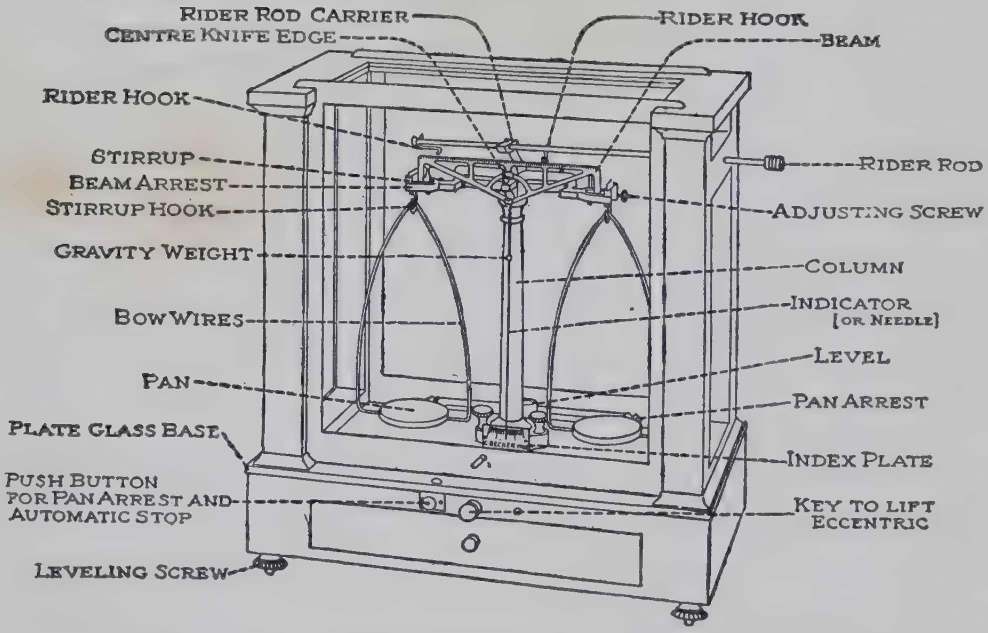

RIDER ROD CARRIER
RIDER HOOK
CENTRE KNIFE EDGE
BEAM
RIDER HOOK
STIRRUP
RIDER ROD
BEAM ARREST
STIRRUP HOOK
ADJUSTING SCREW
GRAVITY WEIGHT
COLUMN
INDICATOR (OR NEEDLE)
BOW WIRES
LEVEL
PAN
PAN ARREST
PLATE GLASS BASE
INDEX PLATE
PUSH BUTTON FOR PAN ARREST AND AUTOMATIC STOP
KEY TO LIFT ECCENTRIC
LEVELING SCREW

Fig. 7. Diagram showing parts of the analytical balance.

lowering of the beam upon the knife-edges dulls them and produces furrows in the bearings, which decrease the sensitivity of the balance. A pan arrest is provided which, when pushed in and turned, releases the pans, leaving them suspended from the stirrup. A long pointer which multiplies the rotational displacement is attached to the beam in order to render small movements of the beam perceptible.

Rules for the Use and Care of the Balance:

1. Use the balance assigned by the instructor.

2. Adopt an attitude of personal responsibility for the condition of your balance; the carelessness of one student may render inaccurate the work of all who use the same balance.

3. Brush the pans and floor of the balance with a camel's-hair brush before starting to weigh any substance.

4. Test the adjustment of the balance before each weighing. The balance is properly adjusted only if the following conditions are fulfilled: (*a*) the balance is level as shown by the spirit level; (*b*) the mechanism for releasing and arresting the beam works freely and smoothly; (*c*) the pan arrests just touch the pans when the beam is lowered; (*d*) the pointer rests at zero when the beam is either released or arrested while the pans are supported; and (*e*) the pointer swings approximately equal distances on either side of zero on the index plate when the beam is set in motion without a load on the pans or with an equal load on each pan. Allowance may be made for a variation of one division on the index scale without adjustment.

5. Do not attempt to adjust the balance. Call an instructor if it requires adjustment.

6. No sample should ever be placed directly upon the balance pan. Solids may be weighed on a watch glass or in a weighing bottle, and liquids must always be weighed in a tightly stoppered bottle. The use of sheets of paper in place of a proper container is not permissible.

7. Hot objects must be allowed to cool to room temperature before placing them inside the balance case, since currents of warm air tend to buoy up one arm of the balance and also to cause that arm to expand in length.

8. Material spilled on the balance pans or floor must be brushed up at once.

9. The balance door must be closed when the final weighing is made.

10. The beam should be set in motion by gently lowering it so that the pans are supported by the pan arrests; the pan arrests are then carefully lowered, and if the beam does not swing, it may be set in motion by means of the rider or by gently fanning one of the pans. Long swings of the pointer are not necessary. The pointer should swing through an amplitude of not less than three divisions or more than six divisions to either side of the zero of the index scale.

11. All weights should be handled with forceps; they should never be touched with the fingers. Place heavy weights in the center of the pan to prevent oscillation.

12. Record all weights directly into your notebook upon completion of a weighing. Before removing weights, check the balance by arresting and releasing the beam and be sure that correct balance has been attained. Remember that a slight error in weighing may render worthless all further analysis of a sample.

13. Before leaving the balance, stop the motion of the beam by means of the pan arrests, raise the beam off the agate knife-edges, be sure the balance is clean, and close the door of the balance case.

Sensitivity of the Analytical Balance. The sensitivity varies inversely with the weight of the beam. The lighter the beam, therefore, the greater the sensitivity. The sensitivity varies with the length of the beam. The sensitivity varies directly with the time period of oscillation. The sensitivity varies inversely with the load on the pans. The sensitivity of a balance may be varied by moving the small gravity weight on the pointer upward or downward, thus changing the position of the center of gravity of the beam. The efficient operation and sensitivity of a balance are dependent upon minimum friction between the agate knife-edges and bearings.

Exercise 1

Object. Determination of the True Zero Point of a Balance.

Procedure. Dust the pans and floor of the balance with a camel's-hair brush. Close the balance door. Gently release the beam and pan arrests, avoiding setting the beam in motion. Cause the beam to swing so that the pointer moves past not more than twelve or less than six divisions of the index scale by carefully touching one arm of the beam with the rider. Allow the pointer to complete two oscillations without taking a reading. Then take three readings of the limit of pointer oscillation on one side of the index scale and two on the other, counting first left then right. Calculate the average reading for each side and the difference between the averages. Divide the difference by 2; the result gives the true zero point of the balance.

The zero point is then computed as follows:

$$\begin{array}{rr} \text{Left} & \text{Right} \\ 5.2 & \\ 5.1 & 4.6 \\ 5.0 & 4.4 \\ 3\overline{)15.3} & 2\overline{)9.0} \\ \hline 5.1 & 4.5\ \text{average} \end{array}$$

$$5.1 - 4.5 = 0.6\ \text{difference}$$

$$\frac{0.6}{2} = 0.3\ \text{divisions to the left}$$

The true zero point is 0.3 of a scale division to the left. Make at least three such zero-point determinations. The results should check within two-tenths of a scale division.

The true zero point of a balance varies from day to day and should be determined each time the balance is used.

Alternate methods of making readings on the index scale might be recommended by the instructor. For example, many analytical chemists prefer to designate the extreme left scale division as zero and the successive divisions to the right as 10, 20, 30, . . . 200, thus making the division at the center of the scale 100. Such a procedure eliminates the necessity of recording decimal points. It should be noted that the average readings of the two limits of the period of oscillation are added together and the sum divided by 2 in calculation of the zero point, when the scale is read according to this alternate procedure.

Exercise 2

Object. Determination of the Sensitivity of a Balance.

Procedure. The sensitivity of a balance is the displacement of the zero point produced by a weight of 1 mg. Determine the true zero, or rest, point of the balance. Place the rider on the 1-mg. division of the beam scale and determine the new rest point on the index scale.

The difference between the two rest points gives the sensitivity of the balance in terms of index scale divisions. Assume that the difference in the rest points is two, then 1 mg. causes a displacement of two scale divisions. Since the index scale can be read to one-tenth of a division and there are 20 one-tenth divisions, the balance is said to be sensitive to 0.05 mg., *i.e.*, the smallest weight that can cause a readable deflection is 0.05 mg.

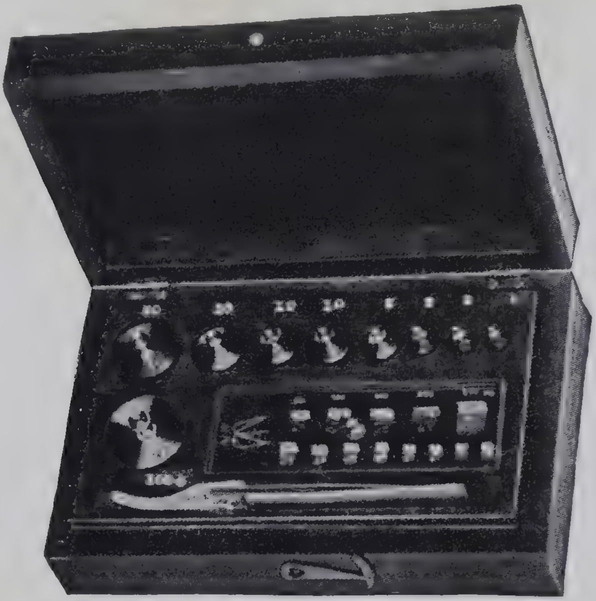


FIG. 8. Set of analytical weights.

Determine the sensitivity of the balance with loads of 10 Gm. on each pan and with 20 Gm. on each pan. Tabulate your results.

WEIGHTS

Figure 8 illustrates a set of analytical weights. Analytical weights may be purchased in graduated sets; a set in which the largest weight is 50 Gm. and the smallest 5 mg. is satisfactory for most analytical work. The 1-Gm. and all larger weights are usually made of brass and plated with gold or coated with lacquer to prevent corrosion. The fractional weights are usually made of platinum, tantalum, or aluminum.

Comparatively accurate weights can be purchased, but an analyst must know that his weights are accurate and should, therefore, always calibrate the weights at least once each year.

Exercise 3

Object. Calibration of a Set of Weights.

Procedure. Clean the set of weights to be calibrated by wiping them thoroughly with a clean cloth and brush the weight box with a stiff brush.

CALIBRATION CHART

Left	Right	Rider reading	Actual weight	Aliquot part	Actual correction
0.005	Rider	+0.0050	0.0050	0.0050	0.0
0.010	0.005	+0.0052	0.0102	0.0100	+0.0002
0.010	0.01	+0.0001	0.0103	0.0100	+0.0003
0.020	0.01 + 0.01	−0.0002	0.0203	0.0200	+0.0003
0.050	0.02 + 0.01 + etc.	+0.0042	0.0500	0.0501	+0.0001
0.100	0.05 + 0.02 + etc.	+0.0045	0.1003	0.1002	+0.0001
0.100	0.05 + 0.02 + etc.	+0.0044	0.1002	0.1002	0.0000
0.200	0.100 + 0.100	+0.0008	0.2013	0.2004	+0.0009
0.500	0.200 + etc.	+0.0044	0.5020	0.5010	+0.0010
1.0	0.500 + etc.	+0.0040	1.0036	1.0019	+0.0017
1.0	0.500 + etc.	+0.0024	1.0020	1.0019	+0.0001
1.0	0.500 + etc.	+0.0034	1.0030	1.0019	+0.0011
2.0	1 + 1	−0.0014	2.0042	2.0038	+0.0004
5.0	2 + 1 + 1 + 1	0.0000	5.0128	5.0096	+0.0032
10.0	5 + 2 + 1 + 1 + 1	−0.0064	10.0192	10.0192	0.0000
10.0	5 + 2 + 1 + 1 + 1	−0.0052	10.0204	10.0192	+0.0012
20.0	10 + 10	−0.0024	20.0372	20.0384	−0.0012
50.0	20 + 10 + 10 + etc.	−0.0032	50.0992	50.0960	+0.0032

Mark duplicate weights by punching one or two dots on them so that they may be readily distinguished. Determine the zero point of the balance. Place the 5-mg. weight on the left pan and the rider on the 5-mg. position on the right arm of the beam scale and determine the zero point again. Transfer the 5-mg. weight to the right-hand pan and place the 10-mg. weight on the left-hand pan and secure perfect balance by means of the rider. Proceed in like manner with the remaining weights. Always place the unknown weights on the left pan and the known weights on the right pan. Tabulate the data according to the illustration on p. 38.

The information secured in the first four columns is all that is required for a calibration of the weights in terms of relative units. This method of calibration is sufficiently accurate for all ordinary quantitative work. The calculation of the actual correction is found by taking the 10-Gm. weight in terms of the preliminary standard and finding the aliquot part of this weight represented by each of the other weights. In our table the actual weight of the 10-Gm. weight in terms of the preliminary standard was found to be 10.0192; the aliquot part of the 5-Gm. weight will then be 10.0192/2 = 5.0096; the aliquot part of the 2-Gm. weight will be 10.0192/5 = 2.0038; and the aliquot part of the 1-Gm. weight, 10.0192/10 = 1.0019; etc. The aliquot part of 10.0192 is represented in the fifth column as calculated above. In the sixth column are given the actual corrections as plus or minus depending on whether the actual weight is greater or less than the aliquot part.

The corrections in tenths of a milligram should be tabulated on a card, and the card fitted tightly into the cover of the weight box.

Example of the Tabulation of Weight Corrections

Weight, Gm.	Correction, 0.1 mg.	Weight, Gm.	Correction, 0.1 mg.
0.005	0	1.0	+17
0.010	+ 2	1.0	+ 1
0.010	+ 3	1.0	+11
0.020	+ 3	2.0	+ 4
0.050	0	5.0	+32
0.100	+ 1	10.0	0
0.100	0	10.0	+12
0.200	+ 9	20.0	−12
0.500	+10	50.0	+32

Riders. Sets of analytical weights may contain 5- or 10-mg. riders or both. It is, of course, essential, in computing a weight from the position of the rider on the beam, that the operator know which type of rider he is using. The type of rider is readily ascertained by observing the rest point when the rider is located at the extreme right division on the beam and a 5- or 10-mg. weight is on the left pan. If a rider at this position is counterpoised by a 10-mg. weight without any significant displacement of the zero point, the rider is a 10-mg. type.

Questions and Problems

1. Why must the qualitative tests precede the assay to obtain accurate data relative to the purity of an official substance?

2. What is a Gooch crucible? Why should asbestos fiber washed with hydrochloric acid be used in preparing this crucible for use in filtration?

3. What is a desiccator? What two agents are used most commonly to dehydrate official samples before assay?

4. What factors affect the sensitivity of a balance?

5. Why are agate knife-edges and agate supports used in balances?

6. Why should the true zero point of a balance be determined before each weighing?

7. Explain how the sensitivity of a balance may be determined with a load of 10 Gm. on each pan.

Chapter 2

GRAVIMETRIC ANALYSIS

THEORY

The student should be familiar with the modern chemical theories which apply in quantitative analysis from the study of general chemistry and qualitative analysis. In quantitative analysis, theoretical considerations must be understood and applied in order to know the full explanation of what takes place in an analysis. A brief review of the fundamentals of the various theories with their quantitative application will be given in conjunction with those procedures to which they apply.

The reactions of quantitative analysis take place in accordance with the established laws and theories of chemistry, *i.e.*, theory of ionization, law of mass action, common ion effect, reversible reactions, and solubility product principle.

Theory of Ionization. The theory of ionization is based on the following postulates:

1. Solutions of electrolytes are dispersions of ions. Strong electrolytes are ionized even when not dissolved. Weak electrolytes undergo ionization on dissolving; thus, when hydrogen chloride gas dissolves in water, the hydrogen chloride molecules dissociate or ionize to form hydrogen ions and chloride ions so that, from one molecule of hydrogen chloride, two ions are formed. The ions thus formed are solvated by interaction with solvent molecules. In water a hydrogen ion, generally represented for convenience as H^+, is hydrated and is more exactly represented as a hydronium ion or H_3O^+.

2. Some of the ions are charged positively (cations), others negatively (anions); the sum of positive and negative charges always being equal, the solution remains electrically neutral. The hydrogen ions are charged positively, and the chloride ions negatively,

the sum of positively charged hydrogen ions being equal to the sum of negatively charged chloride ions; the solution of hydrogen chloride gas remains electrically neutral.

3. The properties of the ions into which the electrolyte dissociates differ from those of the original substance. The hydrogen and chloride ions have properties different from those of the hydrogen chloride molecules, and also from those of hydrogen and chlorine molecules, respectively.

4. The ionization of a weak electrolyte results in an equilibrium because ionization of the molecules of the weak electrolyte is reversible. The composition of the equilibrium mixture depends on the electrolyte, the solvent, and the concentration; thus, electrolytes differ in the extent to which they ionize; some solvents, such as ether and benzene, produce no ionization; in solvents which produce ionization, the greater the dilution the greater the extent of ionization of the electrolyte.

A certain state of equilibrium exists between the ions and undissociated molecules for every degree of dilution. The equilibrium may be represented as follows:

$$HC_2H_3O_2 \rightleftharpoons H^+ + C_2H_3O_2^-$$

when acetic acid is dissolved in water. Since the extent of dissociation is dependent on dilution, the greater the dilution of the solution the more acetic acid molecules will dissociate into ions until, at infinite dilution, it may be regarded as completely ionized. In accordance with the law of mass action, at any dilution, the ratio of the product of the molar concentrations (moles per liter) of hydrogen ions, $[H^+]$, and acetate ions, $[C_2H_3O_2^-]$, to that of unionized acetic acid molecules, $[HC_2H_3O_2]$, is constant, provided temperature is constant.

$$\frac{[H^+][C_2H_3O_2^-]}{[HC_2H_3O_2]} = K$$

Reversible Reactions. Most of the reactions involved in quantitative analysis are of the reversible type. Under certain conditions they may be made to continue to completion, but under other conditions, they may attain equilibrium before completion, resulting in erroneous data pertaining to the substance being analyzed. It is therefore very important to understand what conditions must be satisfied to make the reaction go forward to completion, so that it will be of value in quantitative work. Three general conditions tend to prevent reversal and lead to completion of a reaction: (1) the formation of an insoluble gas; (2) the formation of a sparingly soluble solid; (3) the formation of very slightly ionized molecules.

According to the law of mass action, the rate of a reaction is proportional to the product of the molecular concentrations of the reacting substances. Since the rate of a reaction depends upon the concentration of every substance taking part in the reaction, the point of equilibrium will depend upon the concentration of each of the components of the two opposing reactions. For example, in the reaction, $KNO_3 + H_2SO_4 \rightleftharpoons KHSO_4 + HNO_3$, the rate of the reaction of potassium nitrate with sulfuric acid is expressed by the equation

$$\text{Rate} = [KNO_3] \times [H_2SO_4] \times k$$

where k is a constant which corrects for all factors that affect the rate other than concentration.

The rate of the opposing reaction is expressed by the equation

$$\text{Rate} = [KHSO_4] \times [HNO_3] \times k_1$$

At equilibrium the rates of the two reactions are equal.

$$[KNO_3] \times [H_2SO_4] \times k = [KHSO_4] \times [HNO_3] \times k_1$$

and

$$\frac{[KNO_3] \times [H_2SO_4]}{[KHSO_4] \times [HNO_3]} = \frac{k_1}{k} = K$$

Since k_1 and k are constants, their quotient K is a constant termed the *equilibrium constant.*

At a definite temperature the equilibrium constant is a fixed value for any given reaction irrespective of the concentration of the substances present. If, therefore, the concentration of sulfuric acid is increased, all the other concentrations must change, the concentration of KNO_3 must become less, and that of both $KHSO_4$ and HNO_3

greater to maintain the equilibrium constant, with the result that equilibrium is forced to shift toward the right. In quantitative analysis, an excess of one component is frequently added to cause the reaction to go as nearly to completion as possible.

Solubility Product Principle. The solubility product principle is an application of the law of mass action to equilibriums that generalizes the behavior of difficultly soluble salts in their saturated solutions. Whenever precipitation occurs or is prevented, or solution effected, the conditions to which this principle applies are involved. The principle may be stated as follows: *The product of the concentration of the constituent ions in a saturated solution of a difficultly soluble salt for any given temperature is practically a constant, each concentration being raised to a power equal to the relative number of ions supplied by one molecule of the salt upon dissociating.* For example, a difficultly soluble salt $AmBn$ upon dissociating would furnish a relative number of m cations and n anions:

$AmBn \rightleftharpoons mA^+ + nB^-$ and, using C to designate concentration, the solubility product would be $(C_{A+})^m \times (C_{B-})^n$ = solubility product $AmBn$.

The solubility products of some of the more difficultly soluble salts dealt with in pharmaceutical analysis are given in Table 1.

When a number of different kinds of ions are present in the same solution, the greatest concentration that any one of them can attain is determined by the others. Thus, no great concentration of Ag^+ can be present in a solution in the presence of Cl^-, for the two ions unite to form a precipitate of the difficultly soluble salt, silver chloride. Silver chloride is slightly soluble in water, the solubility being about 0.00001 mole (1.5 mg.) per liter. When this concentration is exceeded, a precipitate of AgCl forms that is in equilibrium with the dissolved silver chloride. When the equilibrium is established, the supernatant liquid is a saturated solution, and the rate at which the solid goes into solution is exactly equal to the rate at which the dissolved salt precipitates. When this condition exists, the following scheme of equilibriums is set up:

$$\underset{\substack{\text{solid}\\\text{ppt.}}}{AgCl} \rightleftharpoons \underset{\substack{\text{dissolved}\\\text{unionized}}}{AgCl} \rightleftharpoons \underset{\substack{\text{dissolved}\\\text{ionized}}}{Ag^+ + Cl^-}$$

The ionization equilibrium expressed according to theory is

$$\frac{[Ag^+] \times [Cl^-]\text{ ionized}}{[AgCl]\text{ unionized}} = K = \text{ionization constant}$$

Since the solution is saturated with AgCl at a given temperature and the concentration of unionized [AgCl] remains constant, it follows that the product $K \times [AgCl]$ also remains constant and that *in a saturated solution of a difficultly soluble salt, the product of the molecular concentrations of its ions is constant.*

TABLE 1. SOLUBILITY PRODUCTS OF SOME IMPORTANT SALTS

Substance	Temperature, °	Ions involved	Solubility product
Aluminum hydroxide	25	$Al^{3+} + 3OH^-$	1×10^{-33}
Barium carbonate	25	$Ba^{++} + CO_3^=$	8.1×10^{-9}
Barium sulfate	25	$Ba^{++} + SO_4^=$	1.1×10^{-10}
Calcium carbonate	25	$Ca^{++} + CO_3^=$	8.7×10^{-9}
Calcium oxalate	25	$Ca^{++} + C_2O_4^=$	2.6×10^{-9}
Lead carbonate	18	$Pb^{++} + CO_3^=$	4.0×10^{-14}
Lead sulfate	18	$Pb^{++} + SO_4^=$	1.1×10^{-8}
Magnesium ammonium phosphate	25	$Mg^{++} + NH_4^+ + PO_4^{3-}$	2.5×10^{-13}
Magnesium hydroxide	18	$Mg^{++} + 2OH^-$	1.5×10^{-11}
Magnesium oxalate	18	$Mg^{++} + C_2O_4^=$	8.8×10^{-5}
Mercuric sulfide	25	$Hg^{++} + S^=$	1×10^{-50}
Mercurous chloride	25	$Hg_2^{++} + 2Cl^-$	1.5×10^{-18}
Silver bromide	25	$Ag^+ + Br^-$	7.7×10^{-13}
Silver chloride	25	$Ag^+ + Cl^-$	1.5×10^{-10}
Silver iodide	25	$Ag^+ + I^-$	1.5×10^{-16}
Silver thiocyanate	25	$Ag^+ + SCN^-$	1.2×10^{-12}

In the calculation of the solubility product, it is customary to express the concentration in terms of moles per liter, *i.e.*, molecular concentrations. A saturated solution of silver chloride contains 1.76×10^{-3} Gm. (0.00176 Gm.) per liter. The molecular weight of silver chloride is 143.34. In terms of molarity, therefore, the solution contains

$$\frac{1.76 \times 10^{-3}}{143.34} = 1.23 \times 10^{-5}$$

mole per liter. At this dilution the dissolved silver chloride may be assumed to be completely ionized, $AgCl \longrightarrow Ag^+ + Cl^-$, so that each mole of silver chloride furnishes 1 mole of silver ions and 1 mole of chloride ions. The solubility product, S.P., is

$$[Ag^+] \times [Cl^-] = (1.23 \times 10^{-5})(1.23 \times 10^{-5}) = 1.5 \times 10^{-10}$$

If the solubility of a compound is known, the solubility product may be calculated; *e.g.*, a saturated solution of silver iodide at 25° contains about 0.00288 mg. of AgI per liter. The molar solubility is equal to the solubility in grams divided by the gram-molecular weight, or

$$\frac{288 \times 10^{-8}}{234.8} = 1.23 \times 10^{-8} \text{ mole per liter}$$

Assuming 100 per cent ionization at this high dilution, the concentrations of Ag^+ and of I^- each equal 1.23×10^{-8}, since each molecule of AgI forms one Ag^+ and one I^- ion. The solubility product is equal to the product of the concentrations of the ions:

$$[Ag^+] \times [I^-] = (1.23 \times 10^{-8})(1.23 \times 10^{-8}) = 1.5 \times 10^{-16}$$

From the above calculation of solubility product, it can be predicted that silver iodide will precipitate if $[Ag^+] \times [I^-]$ becomes greater than 1.5×10^{-16}; that in a supersaturated solution, $[Ag^+] \times [I^-]$ becomes greater than 1.5×10^{-16}; that in a saturated solution $[Ag^+] \times [I^-]$ will be equal to 1.5×10^{-16}; and that to dissolve precipitated AgI, $[Ag^+] \times [I^-]$ must be less that 1.5×10^{-16}.

In the above illustration, the solubility product of silver iodide was calculated from solubility data. Conversely, if the solubility product is known, the solubility of a compound may be calculated. In the table, the solubility product of silver chloride is given as 1.5×10^{-10} at 25°. How much silver chloride will dissolve in 100 cc. of water at the above temperature?

$$\text{S.P.}_{AgCl} = [Ag^+] \times [Cl^-] = 1.5 \times 10^{-10}$$

If $[Ag^+] = x$, then $[Cl^-] = x$, since $[Ag^+] = [Cl^-]$

$$x^2 = 1.5 \times 10^{-10}$$
$$x = 1.22 \times 10^{-5}$$

x is expressed in terms of moles per liter. Since the solubility of AgCl is equal to 1.22×10^{-5} mole per liter, the solubility in grams per 100 cc. is equal to $\frac{1.22 \times 10^{-5}}{10}$ times the gram-molecular weight of AgCl or $1.22 \times 10^{-6} \times 143.34 = 0.000175$ Gm. of AgCl soluble in 100 cc. of water.

The solubility product is thus seen to be an ultimate value attained by the ionic product when equilibrium has been established between the undissolved solid and the difficultly soluble salt in solution. If the product of the concentration of any pair of ions in solution is made to exceed in value the solubility product of the compound formed by their union, precipitation of the compound will take place until the product of the ionic concentration is exactly equal to the solubility product value; when the product of the ionic concentrations is made less than the solubility product value, the compound formed by their union will dissolve until the product of ionic concentrations is equal to the solubility product value.

Common Ion Effect. The equilibrium constant does not change no matter what the concentration of the reacting substances may be. The relative concentration of the reacting substances may change, but there is no change in the equilibrium constant. Thus, when a solution of silver nitrate is added to a solution of sodium chloride, the chloride ion is momentarily present in a concentration such that its ionic product with the silver ion exceeds the solubility product of silver chloride, and the insoluble silver chloride is precipitated:

$$Ag^+ + Cl^- \longrightarrow AgCl\downarrow$$

When an equivalent amount of silver nitrate has been added, and the system has acquired equilibrium, the concentration of silver ions will be exactly equal to the concentration of chloride ions.

If to the supernatant liquid, which is a saturated solution of silver chloride, a small amount of a soluble silver salt or a soluble chloride be added, a slight further precipitation will take place. It follows from the application of the equilibrium representing the ionization constant

$$\frac{[Ag^+] \times [Cl^-]}{[AgCl]} = K$$

that if the concentration of silver ion be increased by the addition of a soluble silver salt, the concentration of chloride ion must decrease and, conversely, that if the concentration of chloride ion be increased by adding a soluble chloride, the concentration of silver ion must decrease since their product remains constant. This decrease in the concentration of the ions in either case can be accomplished only by the union of silver and chloride ions to form insoluble silver chloride forcing the reaction toward completion.

The common ion effect is used frequently in gravimetric pharmaceutical analysis to drive reactions toward completion. Other examples of common ion effect will be discussed in conjunction with the assays in which they occur.

Questions and Problems

1. What are the postulates upon which the theory of ionization is based?

2. What is a reversible reaction?

3. What conditions tend to destroy equilibrium and cause a reaction to go to completion?

4. Define and illustrate the solubility product principle.

5. If 0.124 mg. of barium sulfate dissolve in 50 cc. of water at 25°, express the solubility in terms of molar solubility.

6. From the solubility of $BaSO_4$ given in problem 5, calculate its solubility product assuming 100 per cent ionization.

7. The solubility product of calcium sulfate is approximately 2×10^{-4}. Calculate the amount of calcium sulfate soluble in 500 cc. of water. Assume 100 per cent ionization.

8. Illustrate how common ion effect may be utilized to secure quantitative precipitation.

9. A saturated solution of lead chloride at 20° contains 0.07 mole per liter. This solution is 80 per cent ionized. Calculate the solubility product.

Chapter 3

GRAVIMETRIC METHODS

Gravimetric analysis, the measurement of the weight of a substance in a sample or calculation of the weight of a substance in a sample from the weight of a chemically equivalent amount of some other substance, can be accomplished in various ways. Frequently in pharmaceutical quantitative analysis, the substance to be measured gravimetrically is separated from other substances comprising the sample by physical methods, purified and weighed without chemical change. For example, the quantity of acetanilid in acetanilid tablets is determined by extracting, purifying further by complete removal of solvent, and weighing the acetanilid in a sample of the powdered tablets. When the other components of the sample are such that separation by physical means of the substance being measured is not possible or convenient, chemical reaction is employed to convert the substance to a chemically equivalent amount of some other substance which can be separated, purified, and weighed. Using the known weight relationship between the substance being measured and the substance which is ultimately weighed, the weight of the former is readily calculated from that of a chemically equivalent amount of the latter.

In the general reaction

$$A + B \longrightarrow C + D$$

the weight of either reactant, A or B, can be calculated from the weight of either product, C or D. For example, in the reaction

$$NaCl + AgNO_3 \longrightarrow AgCl + NaNO_3$$

58.45 Gm. of NaCl is required to produce 143.34 Gm. of AgCl, or, for each gram of AgCl produced, the sample must have contained

$$\frac{58.45}{143.34} \text{ (1 Gm. of AgCl)} = 0.4078 \text{ Gm. of NaCl}$$

A chemically equivalent amount of some product can be obtained from a reactant, *i.e.*, the component of the sample being assayed, by any one of various methods: (1) it may be precipitated from solution; (2) it may be a decomposition product resulting from ignition of a compound; (3) it may be deposited on an electrode by electrolysis, etc. The first two methods are considered in this chapter.

Exercise 4

Determination of Chlorine in a Soluble Chloride. The weight of chlorine present in a soluble chloride can be determined gravimetrically by calculation from data obtained by precipitation and weighing of a chemically equivalent amount of silver chloride. A solution of a sample of a soluble chloride is acidified with nitric acid and treated with excess silver nitrate solution. The silver chloride which precipitates is collected on a filter, washed, dried, and weighed. Other substances that form insoluble silver salts must be absent from the sample.

Although the chlorine in official soluble chlorides is generally determined by a titrimetric procedure, page 153, the gravimetric procedure constitutes a classic example of gravimetric analytical technique with which every student should be familiar.

Object. Assay of Sodium Chloride.

Materials Required. 0.25 Gm. of sodium chloride.
5 per cent silver nitrate solution.
Diluted nitric acid.
Asbestos fiber.

Procedure. 1. Accurately weigh two samples of 0.2 to 0.3 Gm. of sodium chloride.

This may be done by weighing the sample tube (weighing bottle) and contents accurately to 0.1 mg., without touching it, entering the weight in the notebook. Hold the weighing bottle directly above a 300- to 400-cc. beaker, labeled 1, remove the stopper, and carefully transfer 0.2 to 0.3 Gm. of the sample to the beaker. Replace the stopper and again weigh the weighing bottle to 0.1 mg., entering the weight in the notebook. The first weight less the second is the weight of sample taken. In like manner weigh a second 0.2- to 0.3-Gm. sample into beaker 2, entering the weights as before. Treat each sample as follows:

2. Completely dissolve the sample in 100 to 125 cc. of distilled water. Add about 1 cc. of diluted nitric acid slowly with stirring. Test the solution by touching a strip of blue litmus paper with the moist stirring rod. If it is not acid in reaction, add sufficient acid to make it so. Measure out 5 cc. in excess of the amount of silver nitrate solution theoretically required to precipitate all of the chlorine as silver chloride. Add the silver nitrate solution in small successive portions, stirring constantly with a suitable glass rod. Cover the beaker with a watch glass, and, with occasional stirring, gradually heat the mixture to the boiling point. Turn down the flame and digest the mixture, without boiling, for 10 min. This treatment coagulates the precipitate, causes it to settle, and leaves the supernatant liquid clear. Add 1 or 2 drops of silver nitrate solution to the hot supernatant liquid to test for complete precipitation. If a precipitate forms, add 5 cc. more silver nitrate solution, stir, allow to settle, and test as before. Set the beakers aside away from direct sunlight to allow the precipitate to settle.

The solution is acidified with nitric acid to prevent the precipitation of other substances insoluble in water but soluble in nitric acid, such as carbonates, oxides, and phosphates. The acid also helps to coagulate any colloidal silver chloride. Too great an excess of nitric acid must not be added, since it exerts some solvent action on silver halides. The acidified solution should not be heated until the silver nitrate has been added, since chlorine may be liberated and lost, thus:

$$\mathrm{NaCl} + \mathrm{HNO_3} \longrightarrow \mathrm{HCl} + \mathrm{NaNO_3}$$
$$6\mathrm{HCl} + 2\mathrm{HNO_3} \longrightarrow 3\mathrm{Cl_2} + 4\mathrm{H_2O} + 2\mathrm{NO}$$

The silver nitrate is added in excess to drive the reaction to completion through common ion effect, assuring complete precipitation:

$$\mathrm{AgNO_3} + \mathrm{NaCl} \longrightarrow \mathrm{AgCl}\downarrow + \mathrm{NaNO_3}$$

The precipitation must be performed away from strong light, since silver chloride is decomposed by sunlight with loss of chlorine, while in diffused light the error from this source is negligible.

The precipitate is digested near the boiling point to coagulate colloidal silver chloride which, if present, would pass through the filter.

3. During intervals in the above procedure, carefully clean and prepare two Gooch crucibles for filtration as previously described. After the crucibles have been properly prepared, heated to constant weight, and weighed, fit them into the suction flask. Be sure that the crucibles can be identified as 1 and 2 by a suitable mark. Decant most of the supernatant liquid into the Gooch crucible, using gentle suction to hasten filtration, guiding the liquid into the crucible by means of a glass rod held against the lip of the beaker. Wash the precipitate three times with 15-cc. portions of hot $0.01N$ nitric acid.

In pouring the clear liquid through the filter, retain as much of the precipitate as possible in the beaker. When the third wash solution has been added to the precipitate, transfer the precipitate into the Gooch crucible using a stream of hot wash solution from the wash bottle and a "policeman" to dislodge all particles that adhere to the sides of the beaker and guide rod. Wash the precipitate on the filter with several small portions of the wash solution, allowing each portion to run through before adding another. Continue washing the precipitate in this manner until 1 to 2 cc. of filtrate give no test for silver ion upon the addition of a few drops of dilute hydrochloric acid solution. When tested in this way, the filtrate should give no greater turbidity than a portion of the wash solution being used tested in the same manner.

4. When the filtrate is shown by the test to be free of silver nitrate, wash the precipitate twice with 5-cc. portions of distilled water to remove most of the nitric acid retained by the precipitate from the former wash solution. Apply strong suction to remove as much liquid from the precipitate as possible. Dry the crucible to constant weight at 110 to 120° in an air bath or electric oven until successive weighings differ by no more than 0.0002 Gm. Allow the crucibles to cool in a desiccator before weighing. The weight of the crucible and precipitate minus the weight of the crucible gives the weight of silver chloride obtained from the sample.

The precipitate is washed to remove soluble salts present in the solution, chiefly sodium nitrate and excess silver nitrate. Hot wash solution increases the solubility of these salts and keeps the silver chloride from passing into the colloidal condition. The solubility of silver chloride increases slightly in hot solution, but this is compensated for by the smaller amount of wash solution required.

Silver chloride is appreciably volatile when ignited, so it should be dried at a relatively low temperature.

OCD TECHNOLOGICAL RESEARCH

The reaction involved in the calculation of the amount of silver nitrate solution to be used and of the purity of the sample is

$$\begin{array}{cccc} AgNO_3 + & NaCl \longrightarrow & AgCl + & NaNO_3 \\ 169.89 & 58.45 & 143.34 & 85.01 \end{array}$$

The equation shows that 1 molecule of sodium chloride reacts with 1 molecule of silver nitrate. From the molecular weights $NaCl = 58.45$ and $AgNO_3 = 169.89$, therefore, the theoretical amount of silver nitrate solution required to precipitate all the chloride ion as silver chloride can be calculated as follows:

$169.89/58.45 = 2.9066$ Gm. of $AgNO_3$ required to precipitate the chlorine from 1 Gm. of NaCl. Since, from the data, 0.3160 Gm. of NaCl was used, then $0.3160 \times 2.9066 = 0.9185$ Gm. of $AgNO_3$ would be required if the sample were pure NaCl. Since the reagent silver nitrate is a 5 per cent solution, each cubic centimeter must contain 0.05 Gm. of $AgNO_3$, and the amount of solution required is $0.9185/0.05 = 18.37$ cc. of 5 per cent solution.

The weight of the sample and the weight of silver chloride obtained from it being known, the percentage purity of the sample or the weight of chlorine in the sample may readily be calculated:

$58.45/143.34 = 0.4078$ Gm. of NaCl equivalent to 1 Gm. of AgCl. Since 1 Gm. of AgCl is equivalent to 0.4078 Gm. of NaCl, then 0.7732 Gm. of AgCl found is equivalent to $0.4078 \times 0.7732 = 0.3153$ Gm. of NaCl. The percentage purity of the sample in terms of NaCl is therefore $0.3153/0.3160 \times 100 = 99.78$ per cent.

The 0.4078 value used in the above calculation is a *gravimetric factor* or a chemical factor by use of which the weight of NaCl in the sample proportionate to the weight of AgCl formed can be readily calculated. The significance of the gravimetric factor should always be borne in mind; *viz.*, the weight of NaCl in the sample is 0.4078 times the weight of the AgCl formed, or each gram of AgCl formed is equivalent to 0.4078 Gm. of NaCl in the sample.

Such a gravimetric factor is included in the official gravimetric assay procedures as outlined in the monographs of the U.S.P. and N.F. and facilitates interpretation of the experimental data. For example, the data of gravimetric procedures are generally interpreted by use of the following formula:

$$\frac{W \times E \times 100}{S} = \text{per cent}$$

where W = weight of the product of a chemical reaction involving the substance being assayed.
E = gravimetric factor.
S = weight of the sample.

Using the data given above, the amount of NaCl in 100 Gm. of sample, *i.e.*, the per cent of NaCl in the sample, is calculated as follows:

$$\frac{0.7732 \times 0.4078 \times 100}{0.3160} = 99.78 \text{ per cent}$$

Sample Data for Gravimetric Determinations

Sample No.__________ Date__________

Determination of Sodium Chloride

Procedure.—Sodium chloride sample precipitated and weighed as silver chloride

Weight	Sample No.	
	1	2
Container + sample.	14.3580	14.0420
Container.	14.0420	13.7315
Sample.	0.3160	0.3105
Crucible + AgCl.	19.0205	18.6409
	19.0201	18.6403
	19.0200	18.6402
Crucible.	18.2470	17.8827
	18.2468	17.8827
Silver chloride.	0.7732	0.7575
Sodium chloride.	0.3153	0.3089
Per cent of sodium chloride.	99.78	99.64
Average per cent.	99.7	

Initials of Analyst______

To calculate the quantity of chlorine present as chloride in the sample, the gravimetric factor

$$\frac{\text{Cl}}{\text{AgCl}} = \frac{35.45}{143.34} = 0.2474 \text{ Gm. of Cl per gram of AgCl}$$

is employed. A similar factor for $CaCl_2$, $CaCl_2 \longrightarrow 2AgCl$, would be

$$\frac{CaCl_2}{2AgCl} = \frac{111}{2(143.34)} = 0.3871 \text{ Gm. of } CaCl_2 \text{ per gram of } AgCl$$

or for $CaCl_2.6H_2O$:

$$\frac{CaCl_2.6H_2O}{2AgCl} = \frac{219.1}{2(143.34)} = 0.7640 \text{ Gm. of } CaCl_2.6H_2O \text{ per gram of } AgCl$$

The gravimetric method for determination of chlorine in a soluble chloride can also be applied in the assay of most soluble bromides, iodides, cyanides, thiocyanates, etc., as well as in the standardization of hydrochloric acid solutions and the estimation of silver compounds. Chlorates, perchlorates, etc., may be determined by this method after reduction to the corresponding halides. Organic halogen compounds usually require preliminary treatment also, *e.g.*, oxidation in solution or combustion by heating with oxidizing agents, before determination of the halogen by the gravimetric method is possible. The student should refer to the monographs of the official substances listed in Table 2 for details of the necessary preliminary treatment. Combustion of sulfobromophthalein sodium in a Parr bomb is illustrative of a procedure often employed in the analysis of organic halogen compounds. The equivalent

TABLE 2. OFFICIAL SUBSTANCES ASSAYED GRAVIMETRICALLY AS SILVER SALTS

Substance	Product of reaction	Gravimetric factor	Official requirement, per cent*
U.S.P.			
Iodopyracet injection	$C_7H_4I_2NO_3Ag$	0.9961	$C_{11}H_{16}I_2N_2O_5$ = 34 to 36 *W/V*
Sulfobromophthalein sodium	AgBr	0.4255	Br = 36 to 39
N.F.			
Acriflavine	AgCl	0.2474	Cl = 13.3 to 15.8
Acriflavine hydrochloride	AgCl	0.2474	Cl = 23.0 to 24.5
Camphorated parachlorophenol	AgCl	0.8968	C_6H_5ClO = 33 to 37
Merbromin	AgBr	0.4255	Br = 18.0 to 21.3
Merbromin solution	AgBr	0.4255	Br = 18.0 to 21.3
Merbromin solution, surgical	AgBr	0.4255	Br = 18.0 to 21.3

* Unless otherwise specified, percentages are expressed *W/W* in all tables.

amount of halide produced by decomposition of the organic compound can be measured gravimetrically as above described, or by an alternate precipitation titrimetric procedure, page 153.

Questions and Problems

1. Why should the solution be acidified with nitric acid?

2. Why add silver nitrate in excess in the precipitation of the chloride?

3. Why is the precipitate washed with water slightly acidified with nitric acid?

4. Why is it best to wash the precipitate by decantation before it is transferred to the Gooch crucible?

5. Why should this determination be carried out away from direct sunlight?

6. Name several official substances other than sodium chloride that may be assayed by an analogous method.

7. What substances, if present, would interfere with the determination of the purity of a soluble chloride?

8. A 0.3056-Gm. sample of a soluble chloride was analyzed gravimetrically for Cl, and 0.7265 Gm. of AgCl was obtained. Calculate the Cl and the NaCl contents of the sample, expressing each in per cent weight to weight.

9. What volume of 10 per cent W/V silver nitrate solution would be necessary to precipitate completely the Cl of 1 Gm. of 50 per cent W/W zinc chloride solution?

10. When is a crucible considered to have been heated to constant weight?

11. A sample of acriflavine lost 7.5 per cent of its weight on drying. The chlorine content of the dried sample was 14.5 per cent. What would be the chlorine content of the undried material? Why is this dual control of purity necessary?

12. Prepare an outline of steps that would probably be necessary in a procedure for the gravimetric analysis of silver.

13. Define and illustrate the terms: gravimetric factor and chemical equivalent.

14. If the solubility of silver chloride in water is 0.00015 Gm. per 100 cc., what is its solubility in $0.01M$ hydrochloric acid?

15. What volume of concentrated hydrochloric acid, sp. gr. 1.19, 36 per cent W/W, would be withdrawn from a weighing pipette so that the sample would yield 0.8500 Gm. of AgCl when treated with silver nitrate in the usual manner?

16. A precipitate of AgCl weighing 0.2432 Gm. was obtained from a 25-cc. sample of a hydrochloric acid solution. Calculate (*a*) the HCl content of the solution in per cent W/V; (*b*) the molarity of the solution; (*c*) the weight of $Ba(OH)_2.8H_2O$ that would be required to neutralize 50 cc. of the solution.

17. A 0.5163-Gm. sample of the silver salt of a monobasic organic acid yielded 0.1690 Gm. of AgCl. Calculate (*a*) the Ag content of the sample in per cent W/W; (*b*) the molecular weight of the organic acid.

18. If a 0.4600-Gm. sample of sodium iodide yielded 0.7200 Gm. of AgI, (*a*) what was the weight of iodine in the sample; (*b*) what was the iodine content of the sample in per cent *W/W*; (*c*) what weight of lead iodide would be formed from 0.5 Gm. of the sample?

19. Calculate (*a*) the molarity; (*b*) the HBr content in per cent *W/V*; (*c*) the volume of 10 per cent *W/V* sodium hydroxide equivalent to 1 cc. of a hydrobromic acid solution, 50 cc. of which yielded 0.2342 Gm. of AgBr.

20. What would be (*a*) the Br content of a 5-Gm. sample; (*b*) the Br content in per cent *W/W*; (*c*) the KBr content in per cent *W/W*, of a sample of potassium bromide, 0.3546 Gm. of which yields 0.4749 Gm. of AgBr?

21. A 0.6102-Gm. sample of potassium chlorate was reduced to chloride and the reaction mixture diluted to 50.00 cc. with water. A 10.00-cc. sample of the resulting solution yielded 0.1425 Gm. of AgCl. Calculate (*a*) the $KClO_3$ content of the sample in per cent *W/W*; (*b*) the weight of Cl formed in the reduction.

22. A 0.5000-Gm. sample of potassium iodate was reduced and the iodide formed converted to silver iodide. When the silver iodide was heated in a stream of chlorine, an equivalent amount of silver chloride was formed and weighed 0.2030 Gm. less than the silver iodide. Calculate the KIO_3 content of the sample in per cent *W/W*.

23. The AgCl obtained from a 0.6500-Gm. sample of sodium chlorate was reduced to 0.6050 Gm. of metallic silver. Calculate the $NaClO_3$ content of the sample in per cent *W/W*.

24. A sample of mixed calcium and silver nitrates weighs 0.3880 Gm.; the silver and calcium are precipitated as mixed oxalates, weighing 0.2800 Gm.; the oxalates are subsequently taken up in nitric acid and electrolyzed, 0.1079 Gm. of metallic silver being collected. Another sample of the mixed nitrates weighing 0.5820 Gm. is treated with excess sodium chloride, giving a precipitate weighing 0.2150 Gm. Calculate the per cent of $AgNO_3$ and the per cent of $Ca(NO_3)_2$ in the sample. What part of the mixed oxalates is $Ag_2C_2O_4$?

25. The phosphorus in a sample weighing 0.50 Gm. is converted to silver phosphate, Ag_3PO_4; this is then converted into AgCl, of which 0.9550 Gm. is formed. Calculate the per cent of phosphorus.

26. A sample consisting only of pure KCl and pure KI weighs 0.4811 Gm.; it is quantitatively converted into KCl and now weighs 0.2982 Gm. Calculate how much KI is present in the original sample.

27. How much NaBr, per cent *W/W*, is in a mixture of pure sodium bromide and pure potassium bromide containing 73.4 per cent Br?

28. A 1.2500-Gm. sample of calcium chloride and calcium bromide yielded a mixture of AgCl and AgBr weighing 2.3740 Gm., and from this, 2.2400 Gm. of AgCl was obtained by heating the mixture in a stream of chlorine. What weight of calcium oxalate, $CaC_2O_4.H_2O$, could be obtained from 0.5 Gm. of the original mixture?

Exercise 5

Determination of Sulfate Ion in a Soluble Sulfate. The sulfate ion in a soluble sulfate may be determined gravimetrically by precipitation as barium sulfate, the precipitate being collected, dried, ignited, and weighed.

Object. Assay of Sodium Sulfate.

Materials Required. 1 Gm. of sodium sulfate.
Barium chloride T.S. (12 per cent).

Procedure. Weigh accurately about 400 mg. of the dried sodium sulfate, obtained by drying about 1 Gm. at 105° for 4 hr., dissolve it in 200 cc. of distilled water, and add 1 cc. of hydrochloric acid. Heat to boiling, and gradually add an excess of hot barium chloride T.S. Heat the mixture for 1 hr. on a water bath, collect the precipitate of barium sulfate on a filter, wash it until free from chloride, dry, ignite, and weigh. The weight of the barium sulfate thus obtained, multiplied by 0.6086, indicates its equivalent of Na_2SO_4.

The solution of the sulfate is acidified with hydrochloric acid to prevent the precipitation of carbonates, etc., which are soluble in hydrochloric acid solution. The acid also increases the solubility of barium sulfate slightly and therefore promotes the growth of large crystals, since small crystals with greater surface area per unit of weight dissolve more rapidly than large ones. The larger crystals with small surface area exposed grow more rapidly than they dissolve. The solution is heated to boiling to expel dissolved CO_2. An excess of hot barium chloride solution is added to ensure complete precipitation and to decrease the solubility of barium sulfate by common ion effect. The mixture is digested on a water bath for 1 hr. to allow the larger crystals to grow at the expense of the smaller ones.

The barium sulfate is directed to be washed on a filter to remove excess $BaCl_2$ and the NaCl formed, but the washing process may be carried out more rapidly by washing once by decantation. The precipitate is then transferred quantitatively onto an ashless filter by the same technique used in Exercise 4 and washed free of chlorides as shown by tests made with silver nitrate solution on 2-cc. portions of the filtrate. The filtrate is always acidified with a drop of nitric acid in carrying out the test for chlorides. Sometimes fine-grained crystalline precipitates will pass through the filter. This may be observed by imparting a rotatory motion to the filtrate, causing any crystals which passed through to gather in the center of the beaker. If such are detected, refilter.

Barium sulfate exhibits the property of "dragging down" other soluble salts when it is precipitated. This property is known as *coprecipitation.* The occluded or coprecipitated salt cannot be removed by ordinary washing. The error from this source is reduced largely by precipitation from hot dilute solutions and by adding the precipitating reagent slowly with continuous stirring. The phenomenon of coprecipitation is particularly liable to occur when barium sulfate is precipitated in the presence of chlorates, nitrates, and the salts of iron, aluminum, chromium, and other trivalent elements.

The precipitate may be dried and ignited by the wet method as described under General Operations (page 29). The equation

$$\underset{142.05}{Na_2SO_4} + BaCl_2.2H_2O \longrightarrow \underset{233.42}{BaSO_4} + 2NaCl + 2H_2O$$

expresses the stoichiometric relationship on which the calculations are based:

$$\frac{142.05}{233.42} = 0.6086 \text{ Gm. of } Na_2SO_4 \text{ equivalent to 1 Gm. of } BaSO_4$$

The gravimetric determination of sulfate ion as barium sulfate is used extensively in pharmaceutical analysis. It may be applied to the estimation of metals that form insoluble sulfates such as barium, lead, etc. The amount of CaO in magnesium carbonate, magnesium oxide, and heavy magnesium oxide, is determined by precipitation of an equivalent amount of $CaSO_4$ under conditions such that $MgSO_4$ remains in solution. Sulfur, free and combined, may be oxidized to sulfate and subsequently precipitated as barium sulfate. The preliminary combustion of sulfobromophthalein sodium and dioctyl sodium sulfosuccinate with oxidizing agents such as sodium peroxide in a Parr bomb before the gravimetric determination as carried out in the assay of sodium sulfate is illustrative of a procedure

TABLE 3. OFFICIAL SUBSTANCES ASSAYED GRAVIMETRICALLY AS SULFATE*

Substance	Gravimetric factor	Official requirement, per cent
U.S.P.		
Sodium sulfate	0.6086	Na_2SO_4 = (n.l.t.) 99
Sulfobromophthalein sodium	0.1374	S = 7.4 to 8.2
Sulfur ointment	0.1374	S = 9.5 to 10.5
Sulfur, precipitated	0.1374	S = (n.l.t.) 99.5
N.F.		
Barium chloride	1.0466	$BaCl_2.2H_2O$ = (n.l.t.) 99
Dioctyl sodium sulfosuccinate	0.1374	S = 7.00 to 7.23
Ichthammol	0.1374	S = (n.l.t.) 10
Potassium guaiacolsulfonate	1.038	$C_7H_7KO_5S$ = (n.l.t.) 96
Sulfur, sublimed	0.1374	S = (n.l.t.) 99.5
Sulfur, washed	0.1374	S = (n.l.t.) 99.5

* The product of the reaction in all instances is $BaSO_4$.
(n.l.t.) = not less than.

frequently used for organic sulfur compounds. Other compounds such as thiosulfates, sulfites, persulfates, etc., may be estimated by the application of similar procedures.

Questions and Problems

1. Why is the solution of soluble sulfate acidified with hydrochloric acid?

2. Why is it necessary to digest the precipitate of barium sulfate previous to filtration?

3. Why should the precipitation of sulfates be carried out in hot, dilute solution?

4. An unknown sample of a soluble sulfate weighing 1.8000 Gm. yielded 0.9000 Gm. of $BaSO_4$. Calculate the percentage of sulfur in the unknown.

5. Calculate the equivalent amounts of each and the percentage purity if the unknown in problem 4 were $MgSO_4$, K_2SO_4, $Al_2(SO_4)_3$, or H_2SO_4.

6. Write the equations representing the reactions that take place in the assay of sulfur ointment.

7. How much $BaSO_4$ would be formed from 1.2000 Gm. of $Na_2SO_4.10H_2O$?

8. What is the per cent of SO_4 in a sample of K_2SO_4 if 0.5000 Gm. yields upon reaction with $BaCl_2$ a precipitate weighing 0.5850 Gm.?

9. Write equations for reactions involved in the assay of dioctyl sodium sulfosuccinate. Calculate the weight of $BaSO_4$ that would be obtained from 200 mg. of a 96 per cent pure sample.

10. Calculate the per cent of S and of metal in each of the following:

a. Na_2SO_4 *b.* $Na_2SO_4.10H_2O$ *c.* $NaHSO_4$
d. $CaSO_4$ *e.* $BaSO_4$ *f.* $PbSO_4$

11. What weight of $BaCl_2.2H_2O$ is required to precipitate the S in 1 Gm. of potassium ferric alum, $K_2SO_4.Fe_2(SO_4)_3$?

12. What volume of barium chloride T.S. would be used in the gravimetric analysis of a 0.4500-Gm. sample of sodium sulfate so that 5 per cent excess of the theoretical amount would be present in the reaction mixture? The sodium sulfate is assumed to be 100 per cent Na_2SO_4.

13. How many grams of barium chloride are necessary to precipitate all the sulfate ion in:

a. 1.4205 Gm. of sodium sulfate?
b. 0.4356 Gm. of potassium sulfate with $10H_2O$?

14. How many grams of Na_2SO_4 are necessary to precipitate quantitatively as sulfate:

a. The Ba in 0.4167 Gm. of $BaCl_2$?
b. The Ca in 0.2220 Gm. of $CaCl_2$?
c. The Pb in 0.2781 Gm. of $PbCl_2$?

Exercise 6

Determination of the Mercury Content of a Mercuric Salt. The mercury is precipitated as sulfide, washed, dried, and weighed.

Object. Assay of Mercury Bichloride.

Materials Required. 0.5 Gm. of mercuric chloride.
1 cc. of hydrochloric acid.
About 70 cc. of alcohol.
About 40 cc. of carbon tetrachloride.

Procedure. 1. "Dry about 500 mg. of Mercury Bichloride over sulfuric acid for 18 hours, weigh accurately, and dissolve it in 300 cc. of warm water, to which 1 cc. of hydrochloric acid has been added. Pass hydrogen sulfide through the solution until the precipitate of mercuric sulfide readily settles, leaving a clear, supernatant liquid."

The hydrogen sulfide gas used in the precipitation should be washed by bubbling it through a gas-washing bottle containing water. The glass delivery tube which passes to the bottom of the precipitation beaker should be attached to the gas-washing bottle by means of a rubber tube in such a manner that it can be removed readily and held over the precipitation beaker while washing any adhering precipitate into the beaker.

The hydrogen sulfide reacts with the mercuric chloride to form a double salt of mercuric sulfide and mercuric chloride at first, and then this double salt is decomposed with the formation of black mercuric sulfide as more hydrogen sulfide is passed into the mixture. Since mercuric sulfide is insoluble in acids, hydrogen sulfide may be employed as the precipitating agent and hydrochloric acid may be added to suppress the concentration of sulfide ion so that metals which form sulfides more soluble than mercuric sulfide and which may be present as impurities will not be precipitated.

2. "Collect the precipitate on counterpoised filters or in a tared Gooch crucible, wash it well with cold water, and finally with 3 portions of about 10 cc. each of alcohol. Then, without suction, wash the precipitate with 10-cc. portions of carbon tetrachloride until after evaporating about 1 cc. of the last filtrate, no visible residue remains. Remove the adhering carbon tetrachloride by washing the precipitate with several portions of 10 cc. each of alcohol, and, after drying in the air, transfer to an oven, and dry at 105° for 2 hours. The weight of mercuric sulfide, multiplied by 1.167, indicates its equivalent in $HgCl_2$."

The precipitate is washed with water to remove soluble sulfides, then with alcohol to remove the water. The carbon tetrachloride, which is added later and is immiscible with water but miscible with alcohol, can permeate the precipitate and dissolve any sulfur formed by the oxidation of hydrogen sulfide. The precipitate is washed with carbon tetrachloride without suction to allow time for the sulfur, which is slowly soluble, to dissolve. After washing the precipitate free of carbon tetrachloride with alcohol, it is dried at 105° because mercuric sulfide is volatile at high temperatures (above 400°).

The reaction that occurs may be represented in its simplest form by the following equation:

$$\underset{271.52}{HgCl_2} + H_2S \longrightarrow \underset{232.68}{HgS} + 2HCl$$

The same procedure can be adapted for the assay of other mercury salts, antimony salts and, after a preliminary oxidative degradation

Table 4. Official Substances Assayed by Precipitation as Sulfide*

Substance	Gravimetric factor	Official requirement, per cent
U.S.P.		
Antimony sodium thioglycollate	1.913	$C_4H_4NaO_4S_2Sb$ = 96 to 101
Antimony sodium thioglycollate, injection	1.913	$C_4H_4NaO_4S_2Sb$ = 93 to 107†
Mercury, ammoniated, ointment	1.083	$HgNH_2Cl$ = 4.5 to 5.5
Mercury, ammoniated, ophthalmic ointment	1.083	$HgNH_2Cl$ = 2.7 to 3.3
Mercury bichloride	1.167	$HgCl_2$ = (n.l.t.) 99.5
N.F.		
Merbromin	0.8622	Hg = 24 to 26.7
Merbromin solution	0.8622	Hg = 24 to 26.7
Merbromin solution, surgical	0.8622	Hg = 24 to 26.7
Mercury bichloride tablets, large poison	1.167	$HgCl_2$ = 420 to 520 mg. per tablet
Mercury bichloride tablets, small poison	1.167	$HgCl_2$ = 110 to 140 mg. per tablet

* The product of reaction is either HgS or Sb_2S_3.

† Per cent of labeled amount.

(n.l.t.) = not less than.

using acid permanganate, for the assay of such organomercury compounds as Merbromin.

Questions and Problems

1. Show how the gravimetric factors for each of the following are derived (*a*) mercury bichloride; (*b*) antimony sodium thioglycollate; (*c*) ammoniated mercury; (*d*) mercury in Merbromin.

2. The presence of hydrochloric acid makes possible the gravimetric analysis of mercury bichloride even in the presence of other ions such as nickel, which react with hydrogen sulfide to form insoluble sulfides. Explain, using equations for the reversible reactions involved and the corresponding expressions for calculation of equilibrium constants.

3. Refer to the official monographs in which the assay procedures of ammoniated mercury ointment and Merbromin are described. Outline and explain the steps in the procedures preliminary to precipitation of the mercuric sulfide.

4. What weight of each of the following should be taken as sample, assuming 100 per cent purity, so that 0.5000 Gm. of HgS would be obtained in a gravimetric analysis: (*a*) mercury bichloride; (*b*) Merbromin; (*c*) mercury bichloride, small poison tablets?

5. Calculate the $HgCl_2$ content of a sample of mercury bichloride, 0.2551 Gm. of which yields 0.1815 Gm. of HgS.

6. What weight of AgCl could be obtained from a sample of mercury bichloride capable of yielding 0.3750 Gm. of HgS?

Exercise 7

Determination of the Aluminum Content and Purity of Alum. The aluminum is precipitated as hydroxide, ignited to oxide, and weighed.

Object. Assay of Alum.

Materials Required. 1 Gm. of alum.
6 Gm. of ammonium chloride.
Ammonia T.S.
Methyl orange T.S.

Procedure. "Dissolve about 1 Gm. of Alum, accurately weighed, and about 5 Gm. of ammonium chloride in 250 cc. of water, and add a few drops of methyl orange, T.S. Heat the solution to boiling, and add a slight excess of ammonia, T.S. until the color is distinctly yellow and then collect the precipitate on a filter, wash thoroughly with a hot solution of ammonium chloride (1 in 100), dry, ignite strongly, and weigh. The weight of the aluminum oxide so obtained, multiplied by 8.891, indicates its equivalent in $AlNH_4(SO_4)_2.12H_2O$ and, multiplied by 9.307, indicates its equivalent in $AlK(SO_4)_2.12H_2O$."

Ammonium chloride is added to the dissolved alum (1) to prevent the formation of the colloidal form of aluminum hydroxide and (2) to prevent the precipitation of other hydroxides of metals, as magnesium, which may be present as impurity. Ammonia water is added to precipitate the aluminum as hydroxide. The ammonia water should be freshly distilled, since upon standing in glass containers it dissolves silica, the presence of which leads to high results, the silica being precipitated and retained in the precipitate. The ammonia water should be added in very slight excess, since aluminum hydroxide is slightly soluble in strong solutions of ammonia. The precipitation is conducted in boiling solution to convert any colloidal aluminum hydroxide into large particles and to secure a coarse-grained precipitate. The solution should not be boiled after complete precipitation, for ammonium salts become acid in reaction upon prolonged boiling due to loss of ammonia, and the resulting acid solution would dissolve some aluminum hydroxide. Aluminum hydroxide forms a slimy precipitate difficult to wash. The precipitate ignited by the wet method should be heated strongly,

Table 5. Official Substances Assayed by Ignition to Oxide

Substance	Product of reaction	Gravimetric factor	Official requirement, per cent
U.S.P.			
Aluminum acetate solution	Al_2O_3	.....	Al_2O_3 = 1.20 to 1.45 W/V
Aluminum hydroxide gel	Al_2O_3	.....	Al_2O_3 = 3.6 to 4.4
Aluminum hydroxide gel, dried	Al_2O_3	.....	Al_2O_3 = (n.l.t.) 50
Aluminum subacetate solution	Al_2O_3	.....	Al_2O_3 = 2.30 to 2.60
Aluminum sulfate	Al_2O_3	6.537	$Al_2(SO_4)_3.18H_2O$ = 99.5 to 112
Bismuth subcarbonate	Bi_2O_3	.....	Bi_2O_3 = (n.l.t.) 90
Bismuth subsalicylate	Bi_2O_3	.....	Bi_2O_3 = 62 to 66
Zinc oxide ointment	ZnO	.....	ZnO = 18.5 to 21.5
Zinc oxide paste	ZnO	.....	ZnO = 24 to 26
Zinc sulfate	ZnO	1.984	$ZnSO_4$ = 55.6 to 61.0
N.F.			
Alum (ammonium)	Al_2O_3	8.894	$AlNH_4(SO_4)_2.12H_2O$ = (n.l.t.) 99.5
Alum (potassium)	Al_2O_3	9.307	$AlK(SO_4)_2.12H_2O$ = (n.l.t.) 99.5
Alum, exsiccated (ammonium)	Al_2O_3	4.652	$AlNH_4(SO_4)_2$ = (n.l.t.) 96.5
Alum, exsiccated (potassium)	Al_2O_3	5.066	$AlK(SO_4)_2$ = (n.l.t.) 96.5
Aluminum chloride	Al_2O_3	4.737	$AlCl_3.6H_2O$ = (n.l.t.) 95
Aluminum chloride solution	Al_2O_3	4.737	$AlCl_3.6H_2O$ = 22.5 to 27.5
Bismuth magma	Bi_2O_3	.....	Bi_2O_3 = 5.2 to 5.8
Bismuth subgallate	Bi_2O_3	.....	Bi_2O_3 = 52 to 57
Bismuth subnitrate	Bi_2O_3	.....	Bi_2O_3 = (n.l.t.) 79
Manganese citrate, soluble	Mn_3O_4	2.373	$C_{12}H_{10}Mn_3O_{14}$ = 48 to 52
Zinc chloride	ZnO	1.675	$ZnCl_2$ = (n.l.t.) 95
Zinc oxide paste with salicylic acid	ZnO	.....	ZnO = 23.5 to 25.5
Zinc phenolsulfonate	ZnO	5.059	$C_{12}H_{10}O_8S_2Zn$ = 73.7 to 77.4

(n.l.t.) = not less than.

preferably over a blast lamp, after the ash has become white to convert any traces of basic aluminum sulfate to the oxide:

$$\underset{2 \times 453.33}{2AlNH_4(SO_4)_2.12H_2O} + 6NH_4OH \longrightarrow 2Al(OH)_3 + 4(NH_4)_2SO_4 + 24H_2O$$

$$2Al(OH)_3 \longrightarrow \underset{101.94}{Al_2O_3} + 3H_2O$$

Since 2(453.33) Gm. of $AlNH_4(SO_4)_2.12H_2O$ are equivalent to 101.94 Gm. of Al_2O_3, 1 Gm. of Al_2O_3 is equivalent to

$$\frac{2(453.33)}{101.94} = 8.894 \text{ Gm.}$$

of $AlNH_4(SO_4)_2.12H_2O$.

Calculate the purity of the alum in terms of $AlNH_4(SO_4)_2.12H_2O$ (ammonium alum) or $AlK(SO_4)_2.12H_2O$ (potassium alum).

Other official substances assayed on the basis of the weight of oxide obtained include oxides and salts of bismuth, zinc, and manganese as indicated in Table 5.

Questions and Problems

1. Why is ammonium chloride added to the alum solution previous to precipitation?

2. Why should a large excess of ammonium hydroxide be avoided?

3. Indicate by reactions how ammonium salts in solution may become acid in reaction upon prolonged boiling.

4. If an unknown sample of alum yields 0.2240 Gm. of Al_2O_3 upon assay, to how much $AlNH_4(SO_4)_2.12H_2O$, $AlNH_4(SO_4)_2$, and $AlK(SO_4)_2.12H_2O$, respectively, is the Al_2O_3 equivalent?

5. Write equations for the reactions that take place in the assay of zinc chloride.

6. How much pure zinc sulfate would be required to form 0.8920 Gm. of ZnO?

7. A sample of soluble manganese citrate weighing 0.5621 Gm. yielded 0.2741 Gm. of Mn_3O_4. What per cent of manganese did the sample contain?

8. A sample of alum, $AlK(SO_4)_2.12H_2O$, weighing 1 Gm. is assayed for both Al and S. It forms $BaSO_4$ weighing 0.9336 Gm. and Al_2O_3 weighing 0.1019 Gm. Calculate the per cent of sulfur and the per cent of aluminum in the sample.

Exercise 8

Determination of the Magnesium in a Magnesium Salt. The magnesium is precipitated as magnesium ammonium phosphate, washed, dried, ignited to pyrophosphate, and weighed.

Object. Assay of Magnesium Sulfate.

Materials Required. 0.3 Gm. magnesium sulfate.
1 cc. of hydrochloric acid.
1.5 Gm. of dibasic ammonium phosphate.
Ammonia T.S.

Procedure. 1. "Weigh accurately about 300 mg. of the ignited Magnesium Sulfate obtained in the test for *Loss on ignition*, and dissolve it in 100 cc. of water and 1 cc. of hydrochloric acid. To the solution add 1.5 Gm. of dibasic ammonium phosphate, dissolved in a small amount of water, and mix well. Add, dropwise, with constant stirring, ammonia T.S. until the solution has a distinct odor of ammonia. Add 40 cc. of ammonia T.S. and allow the mixture to stand for at least 4 hours."

When ammonium phosphate is dissolved in water, the phosphoric acid ionizes in three stages which may be represented by the following equations:

$$H_3PO_4 \rightleftharpoons H^+ + H_2PO_4^-$$
$$H_2PO_4^- \rightleftharpoons H^+ + HPO_4^=$$
$$HPO_4^= \rightleftharpoons H^+ + PO_4^{3-}$$

The degree of ionization becomes less for each successive stage. The relative amount of PO_4^{3-}, $HPO_4^=$, $H_2PO_4^-$, and H_3PO_4 in equilibrium with each other depends upon the hydrogen ion concentration of the solution. In a basic solution, the PO_4^{3-} and $HPO_4^=$ ions predominate. Each of the above species of phosphate ion which exist in the solution of phosphate reacts with Mg^{++} ions to form the corresponding magnesium salts, all of which are more or less insoluble in water. Thus there may be formed $Mg_3(PO_4)_2$, $MgHPO_4$, and $Mg(H_2PO_4)_2$. The addition of a solution of NH_4OH represses the concentration of $H_2PO_4^-$ ions and prevents the formation of $Mg(H_2PO_4)_2$. In the presence of the ammonium ion, the salt is precipitated as $MgNH_4PO_4.6H_2O$. If the solution is too basic, $Mg(OH)_2$ may be precipitated. The precipitation of both $Mg_3(PO_4)_2$ and $Mg(OH)_2$ is prevented by the presence of ammonium salts which, through common ion effect, act as a buffer and prevent the OH ion concentration from becoming too great. The hexahydrate, $MgNH_4PO_4.6H_2O$, forms in relatively coarse crystals on standing for three or more hours at room temperatures. At temperatures above 60° the monohydrate, $MgNH_4PO_4.H_2O$, is formed.

2. "Filter and wash the precipitate with small quantities of a mixture of 1 volume of ammonia T.S. and 3 volumes of water until free from sulfate. Dry, and ignite the precipitate to constant weight. The weight of the magnesium pyrophosphate ($Mg_2P_2O_7$), multiplied by 1.082, gives the equivalent weight of $MgSO_4$."

The precipitate is washed with aqueous ammonia solution until free of sulfates. In testing the filtrate for sulfates, acidify with nitric acid and add barium chloride test solution. Upon ignition, the magnesium ammonium phosphate is converted into the pyrophosphate as follows:

$$2MgNH_4PO_4.6H_2O \rightarrow Mg_2P_2O_7 + 2NH_3 + 13H_2O$$

For the purpose of making calculations, the equation may be represented as

$$\begin{array}{cc} 2MgSO_4 \longrightarrow & Mg_2P_2O_7 \\ 2(120.39) & 222.6 \end{array}$$

From this stoichiometric relationship, it is evident that each gram of $Mg_2P_2O_7$ is equivalent to $2(120.39)/222.6 = 1.082$ Gm. of $MgSO_4$.

The same precipitate, $MgNH_4PO_4.6H_2O$, results when a soluble phosphate is treated with magnesia mixture T.S., *i.e.*, a mixture of $MgCl_2$, NH_4Cl, and NH_4OH. The $Mg_2P_2O_7$ obtained on ignition is a measure of the soluble phosphate in the sample. The application of analogous procedures for determination of manganese salts and the indirect determination of citric acid in raspberry juice is indicated in Table 6.

TABLE 6. OFFICIAL SUBSTANCES ASSAYED GRAVIMETRICALLY AS PYROPHOSPHATE*

Substance	Gravimetric factor	Official requirement, per cent
U.S.P.		
Raspberry juice (citric acid) . .	1.258	$C_6H_8O_7.H_2O$ = (n.l.t.) 1.5 *W/V*
Magnesium sulfate	1.082	$MgSO_4$ = 99.5
N.F.		
Manganese glycerophosphate.	1.586	$C_3H_7MnO_6P$ = 98
Sodium phosphate solution . .	1.276	Na_2HPO_4 = 37 to 41 *W/V*

* The product of reaction in all instances is $Mg_2P_2O_7$ except for manganese glycerophosphate, $Mn_2P_2O_7$.

(n.l.t.) = not less than.

A number of official preparations containing magnesium compounds that were formerly assayed by conversion to equivalent amounts of $Mg_2P_2O_7$ as above described are now assayed by a procedure involving the use of an organic precipitant. The high specificity of organic precipitants, especially when the pH of the reaction mixture is controlled, makes possible qualitative and quantitative analyses of certain ions even in the presence of other ions that interfere in analytical procedures in which inorganic precipitating reagents are used. Certain organic precipitants should be familiar from a study of qualitative analysis; *e.g.*, dimethylglyoxime, cupferron, etc. The reagent, 8-hydroxyquinoline or oxine, selectively forms a very insoluble precipitate with Mg^{++}, $Mg(C_9H_6ON)_2 \cdot 2H_2O$, in a mixture buffered with ammonium chloride and stronger ammonia T.S. This reaction is the basis of the procedure for the quantitative analysis of a number of official preparations as indicated in Table 7.

TABLE 7. OFFICIAL SUBSTANCES ASSAYED AS 8-HYDROXYQUINOLATES*

Substance	Gravimetric factor	Official requirement, per cent
U.S.P.		
Magnesium citrate solution	0.1157†	$MgO = 1.6$ to $1.9W/V$
N.F.		
Magnesium hydroxide	0.1673	$Mg(OH)_2 = 95$
Magnesium hydroxide tablets	0.1673	$Mg(OH)_2 = 93$ to 107‡
Magnesium sulfate injection	0.3453	$MgSO_4 = 46$ to 53§
Sodium bicarbonate and magnesium oxide powder	0.1157	$MgO = 90$ to 110‡
Sodium bicarbonate and magnesium oxide tablets	0.1157	$MgO = 90$ to 110‡

* The product of reaction in all instances is $Mg(C_9H_6NO)_2.2H_2O$.

† The factor given in official procedure is 4.628 because a ¼₀ aliquot part is used.

‡ Per cent of labeled amount.

§ Per cent of labeled amount of $MgSO_4.7H_2O$.

In Table 5 are listed the official bismuth preparations that are assayed by conversion to an equivalent amount of Bi_2O_3. Other official bismuth preparations are assayed by precipitation, etc., of an equivalent amount of bismuth phosphate, $BiPO_4$, and these are given in Table 8.

Calcium salts react quantitatively with ammonium oxalate to form the very insoluble calcium oxalate. However, because of the

TABLE 8. OFFICIAL SUBSTANCES ASSAYED GRAVIMETRICALLY AS PHOSPHATE

Substance	Gravimetric factor	Official requirement, per cent
U.S.P.		
Bismuth potassium tartrate	0.6875	Bi = 60 to 64
Bismuth potassium tartrate injection	0.6875	Bi = 57 to 66 W/V*
Bismuth subsalicylate injection	0.6875	Bi = 53 to 62 W/V*
N.F.		
Bismuth subcarbonate tablets	0.7664	Bi_2O_3 = 83 to 97*
Bismuth subgallate tablets	0.7664	Bi_2O_3 = 48 to 61*
Bismuth subnitrate tablets	0.7664	Bi_2O_3 = 73 to 85*

* Per cent of the labeled amount of the salt.

difficulty of obtaining a uniform product and for the reason of convenience, the precipitated calcium oxalate is generally determined titrimetrically rather than gravimetrically. Calcium glycerophosphate is assayed, nevertheless, by conversion to an equivalent amount of calcium oxalate, $CaC_2O_4.H_2O$, which is collected, dried, and weighed.

The assay of gold sodium thiosulfate and sterile gold sodium thiosulfate is quite readily accomplished because of the ease of reduction of gold in combination to an equivalent amount of elemental gold. After acidification of a solution of a sample, the reducing action of the thiosulfate ion results in precipitation of the gold.

In certain substances, a loss in weight can be used as basis for analysis; *e.g.*, conversion of silicon dioxide, SiO_2, to silicon tetrafluoride, SiF_4, which is a gas; and the volatization of camphor by heating in a stream of CO_2 in the assay of camphor injection and camphor ointment.

The analytical control of organic medicinal agents by gravimetric methods is indicated, in a few instances, in some of the preceding tables. Frequently the organic constituent of an official preparation is isolated and purified by physical procedures and without any permanent chemical change, for example, by extraction or by evaporation of solvent. It may be noted also that no permanent change in composition takes place in the assay of colloidal silver chloride and colloidal silver iodide. In various ways, the colloid is discharged or the colloid stabilizing agent is inactivated, so that the silver halide can be collected on a filter, dried, and weighed.

A list of official substances assayed gravimetrically by separating, purifying, and weighing an organic medicinal agent without any permanent change in composition appears in Table 9. Such proce-

Table 9. Official Substances Assayed Gravimetrically without Chemical Change

Substance	Official Requirement, Per Cent
U.S.P.	
Acetanilid tablets	C_8H_9NO = 94 to 106*
Acetophenetidin tablets	$C_{10}H_{13}NO_2$ = 94 to 106*
Aminopyrine tablets	$C_{13}H_{17}N_3O$ = 94 to 106*
Barbital tablets	$C_8H_{12}N_2O_3$ = 94 to 106*
Bishydroxycoumarin	$C_{19}H_{12}O_6$ = (n.l.t.) 98
Bishydroxycoumarin capsules	$C_{19}H_{12}O_6$ = 90 to 110*
Bishydroxycoumarin tablets	$C_{19}H_{12}O_6$ = 93 to 107*
Collodion	Pyroxylin = (n.l.t.) 5
Congo red injection	$C_{32}H_{22}N_6Na_2O_6S_2$ = 95 to 110*
Erythrityl tetranitrate tablets	$C_4H_6N_4O_{12}$ = 93 to 107*
Estrone injection	Estrone = 90 to 115*
Ethisterone tablets	$C_{21}H_{28}O_2$ = 90 to 110*
Mannitol hexanitrate tablets	$C_6H_8N_6O_{18}$ = 93 to 107*
Pentylenetetrazol injection	$C_6H_{10}N_4$ = 95 to 105*
Phenobarbital elixir	$C_{12}H_{12}N_2O_3$ = 0.37 to 0.43 *W/V*
Phenobarbital tablets	$C_{12}H_{12}N_2O_3$ = 94 to 106*
Sulfobromophthalein sodium injection	$C_{20}H_8Br_4Na_2O_{10}S_2$ = 94 to 106*
N.F.	
Acetophenetidin and phenyl salicylate tablets	Acetophenetidin = 90 to 110*
Aminopyrine elixir	$C_{13}H_{17}N_3O$ = 3.7 to 4.3 *W/V*
Amobarbital elixir	$C_{11}H_{18}N_2O_3$ = 0.417 to 0.462 *W/V*
Amobarbital tablets	$C_{11}H_{18}N_2O_3$ = 94 to 106*
Barbital elixir	$C_8H_{12}N_2O_3$ = 3.2 to 3.8 *W/V*
Ephedrine sulfate and phenobarbital capsules	$C_{12}H_{12}N_2O_3$ = 91 to 109*
Merbromin solution	$C_{20}H_8Br_2HgNa_2O_6$ = 1.8 to 2.2 *W/V*
Merbromin solution, surgical	$C_{20}H_8Br_2HgNa_2O_6$ = 1.8 to 2.2 *W/V*
Santonin tablets	$C_{15}H_{18}O_3$ = 92.5 to 107.5*
Santonin and mild mercurous chloride tablets	$C_{15}H_{18}O_3$ = 92.5 to 107.5*

* Per cent of labeled amount.
(n.l.t.) = not less than.

dures are frequently applied to tablets containing organic medicinal agents. Before extraction of the organic medicinal agent, the sample of the powdered tablets is frequently washed with petroleum benzin to remove substances such as binders and lubricants that would be extracted along with the organic medicinal agent by the solvent, chloroform or ether, which is used subsequently. If the organic medicinal agent is basic, *e.g.*, aminopyrine in aminopyrine tablets, or acidic, *e.g.*, barbital in barbital tablets, it is first extracted with an aqueous solution of an acid or base to effect separation from the neutral substances that might be present. The aqueous solution

TABLE 10. OFFICIAL SUBSTANCES ASSAYED GRAVIMETRICALLY BY CONVERSION TO FREE ACID

Substance	Product of reaction	Gravimetric factor	Official requirement, per cent
U.S.P.			
Barbital sodium	$C_8H_{12}N_2O_3$	1.119	$C_8H_{11}N_2NaO_3$ = (n.l.t.) 98.5
Bartital sodium tablets	$C_8H_{12}N_2O_3$	1.119	$C_8H_{11}N_2NaO_3$ = 94 to 106*
Diphenylhydantoin sodium	$C_{15}H_{12}N_2O_2$	1.087	$C_{15}H_{11}N_2NaO_2$ = (n.l.t.) 98.5
Diphenylhydantoin sodium capsules	$C_{15}H_{12}N_2O_2$	1.087	$C_{15}H_{11}N_2NaO_2$ = 93 to 107*
Fluorescein sodium	$C_{20}H_{12}O_5$	1.132	$C_{20}H_{10}Na_2O_5$ = (n.l.t.) 98.5
Iodophthalein sodium	$C_{20}H_{10}I_4O_4$	.....	$C_{20}H_{10}I_4O_4$ = (n.l.t.) 85
Pentabarbital sodium	$C_{11}H_{18}N_2O_3$	1.097	$C_{11}H_{17}N_2NaO_3$ = (n.l.t.) 98.5
Pentobarbital sodium capsules	$C_{11}H_{18}N_2O_3$	1.097	$C_{11}H_{17}N_2NaO_3$ = 90 to 105*
Pentabarbital sodium injection	$C_{11}H_{18}N_2O_3$	1.097	$C_{11}H_{17}N_2NaO_3$ = 90 to 105*
Pentobarbital sodium sterile	$C_{11}H_{18}N_2O_3$	1.097	$C_{11}H_{17}N_2NaO_3$ = (n.l.t.) 98.5
Pentabarbital sodium tablets	$C_{11}H_{18}N_2O_3$	1.097	$C_{11}H_{17}N_2NaO_3$ = 90 to 105*
Phenobarbital sodium	$C_{12}H_{12}N_2O_3$	1.095	$C_{12}H_{11}N_2NaO_3$ = (n.l.t.) 98.5
Phenobarbital sodium injection	$C_{12}H_{12}N_2O_3$	1.095	$C_{12}H_{11}N_2NaO_3$ = 90 to 105*
Phenobarbital sodium sterile	$C_{12}H_{12}N_2O_3$	1.095	$C_{12}H_{11}N_2NaO_3$ = (n.l.t.) 98.5
Phenobarbital sodium tablets	$C_{12}H_{12}N_2O_3$	1.095	$C_{12}H_{11}N_2NaO_3$ = 90 to 105*
Thiopental sodium	$C_{11}H_{18}N_2O_2S$	1.094	$C_{11}H_{17}N_2NaO_2S$ = (n.l.t.) 97
Thiopental sodium sterile	$C_{11}H_{18}N_2O_2S$	1.094	$C_{11}H_{17}N_2NaO_2S$ = (n.l.t.) 91.7
N.F.			
Amobarbital sodium	$C_{11}H_{18}N_2O_3$	.....	$C_{11}H_{18}N_2O_3$ = 90.0 to 91.6
Amobarbital sodium capsules	$C_{11}H_{18}N_2O_3$	1.097	$C_{11}H_{17}N_2NaO_3$ = 90 to 105*
Amobarbital sodium sterile	$C_{11}H_{18}N_2O_3$	.....	$C_{11}H_{18}N_2O_3$ = 90.0 to 91.6
Pamaquine naphthoate	Acid†	.....	Acid† = 53 to 57
Pentobarbital elixir	$C_{11}H_{18}N_2O_3$	....	$C_{11}H_{18}N_2O_3$ = 0.33 to 0.40 *W/V*

* Per cent of labeled amount.

† Methylene-bis-β-hydroxynaphthoic acid.

(n.l.t.) = not less than.

of the salt of the organic medicinal agent is then made alkaline or acidic as the case may be, and the liberated organic base or acid extracted with chloroform or ether. Bisdihydroxycoumarin, precipitated from alkaline solution by acidification, can be filtered, and subsequent extraction is not necessary. If stearic acid or stearates are present, an organic medicinal agent which is acidic (phenobarbital) cannot be extracted with sodium hydroxide solution because sodium stearate would be extracted along with the salt of the organic acid. In such a case, saturated solution of barium hydroxide is used and the insoluble barium stearate can be removed by filtration.

The active ingredient of a number of official preparations is a salt of an organic acid. When a solution of a sample of such a preparation is acidified with a strong acid such as hydrochloric acid, the organic acid is liberated and may be collected on a filter, *e.g.*, pamaquine naphthoate, or extracted with an immiscible solvent such as ether or chloroform. The analysis of official preparations for salts of organic bases may be carried out in an analogous manner; *i.e.*, the organic base can be liberated by sodium hydroxide and collected on a filter, *e.g.*, dibucaine hydrochloride, or extracted with ether or

TABLE 11. OFFICIAL SUBSTANCES ASSAYED GRAVIMETRICALLY BY CONVERSION TO FREE BASE

Substance	Product of reaction	Gravimetric factor	Official requirement, per cent
U.S.P.			
Chloroguanide hydrochloride	$C_{11}H_{16}ClN_5$	1.144	$C_{11}H_{16}ClN_5.HCl$ = (n.l.t.) 98
Chloroguanide hydrochloride tablets	$C_{11}H_{16}ClN_5$	1.144	$C_{11}H_{16}ClN_5.HCl$ = 93 to 107*
Chloroquine phosphate	$C_{18}H_{26}ClN_3$	1.613	$C_{18}H_{26}ClN_3.2H_3PO_4$ = (n.l.t.) 98
Chloroquine phosphate tablets	$C_{18}H_{26}ClN_3$	1.613	$C_{18}H_{26}ClN_3.2H_3PO_4$ = 93 to 107*
Eucatropine hydrochloride	$C_{17}H_{25}NO_3$	1.125	$C_{17}H_{25}NO_3.HCl$ = (n.l.t.) 97
Pentaquine phosphate	$C_{18}H_{27}N_3O$	1.326	$C_{18}H_{27}N_3O.H_3PO_4$ = (n.l.t.) 97.5
Pentaquine phosphate tablets	$C_{18}H_{27}N_3O$	1.326	$C_{18}H_{27}N_3O.H_3PO_4$ = 94 to 106*
Tetracaine hydrochloride	$C_{15}H_{24}N_2O_2$	1.134	$C_{15}H_{24}N_2O_2.HCl$ = (n.l.t.) 98.5
N.F.			
Dibucaine hydrochloride	$C_{20}H_{29}N_3O_2$	.....	$C_{20}H_{29}N_3O_2$ = 88.5 to 90.5
Phenacaine hydrochloride	$C_{18}H_{22}N_2O_2$	.....	$C_{18}H_{22}N_2O_2$ = 87.5 to 90.5

* Per cent of labeled amount.

(n.l.t.) = not less than.

other solvent. Official preparations assayed for salts of organic acids or bases are summarized in Tables 10 and 11. Specific type methods are also of interest in this connection (pages 115 to 118).

Organic medicinal agents can sometimes be converted quantitatively to derivatives by reactions involving some functional group such as amino, carboxyl, aldehyde or ketone carbonyl, phenolic hydroxyl, etc. Insoluble salts formed by reaction of amino or carboxyl groups may be included in this category. In certain instances it is also possible to obtain uniform substitution products of organic medicinal agents quantitatively, *e.g.*, phenolphthalein tablets. The number of organic medicinal agents that can be analyzed in this way is limited because of the reversible nature of the reactions involved, simultaneous formation of products of side reactions, and other reasons. A list of official substances assayed by such procedures is given in Table 12.

TABLE 12. OFFICIAL SUBSTANCES ASSAYED GRAVIMETRICALLY BY CONVERSION TO DERIVATIVES OR SUBSTITUTION PRODUCTS

Substance	Product of reaction	Gravimetric factor	Official requirement, per cent
U.S.P.			
Diethylstilbestrol	Diacetate	0.7615	$C_{18}H_{20}O_2$ = (n.l.t.) 98.5
Penicillin G potassium	*N*-ethyl-piperidinium salt	0.8323	$C_{16}H_{17}KN_2O_4S$ = (n.l.t.) 85
Penicillin G sodium	*N*-ethyl-piperidinium salt	0.7965	$C_{16}H_{17}NaN_2O_4S$ = (n.l.t.) 85
Progesterone injection	2,4-dinitrophenylhydrazone	0.466	$C_{21}H_{30}O_2$ = 90 to 110*
Tripelennamine hydrochloride	Dipicrate	0.4090	$C_{16}H_{21}N_3.HCl$ = (n.l.t.) 98
Tripelennamine hydrochloride tablets	Dipicrate	0.4090	$C_{16}H_{21}N_3.HCl$ = 93 to 107
N.F.			
Camphor spirit	2,4-dinitrophenylhydrazone	0.4580	$C_{10}H_{16}O$ = 9 to 11*W/V*
Camphorated parachlorophenol	2,4-dinitrophenylhydrazone	0.4580	$C_{10}H_{16}O$ = 63 to 67
Methylene blue	Perchlorate	0.8333	$C_{16}H_{18}ClN_3S$ = (n.l.t.) 98.5
Phenolphthalein tablets	$C_{20}H_{10}I_4O_4$	0.3872	$C_{20}H_{14}O_4$ = 92.5 to 107.5*

* Per cent of labeled amount.
(n.l.t.) = not less than.

Questions and Problems

1. Give equations, indicating all reagents and conditions, for the chemical reactions involved in the formation of $Mg_2P_2O_7$ from (*a*) magnesium sulfate; (*b*) sodium phosphate in sodium phosphate solution.

2. Give the composition or chemical formula of each of the following reagents and state the use of each in gravimetric analysis: (*a*) magnesia mixture T.S.; (*b*) dibasic ammonium phosphate; (*c*) 8-hydroxyquinoline T.S.; (*d*) dinitrophenylhydrazine T.S.

3. A 5.00-cc. sample of sodium phosphate solution was diluted with water to a volume of 100.00 cc. A 10.00-cc. sample of the resulting solution was treated with magnesia mixture T.S. as directed in the official monograph. By ignition of the magnesium ammonium phosphate precipitate, 0.1515 Gm. of magnesium pyrophosphate was obtained. Calculate the Na_2HPO_4 content of the sodium phosphate solution in per cent *W/V*. Does the sample conform to official specifications? If the specific gravity were 1.39, what would be the Na_2HPO_4 content in per cent *W/W*?

4. Calculate the weight of magnesium hydroxyquinolate that would be obtained in the analysis of magnesium citrate solution containing magnesium citrate equivalent to 1.85 per cent *W/V* MgO. Note that in this assay a one-twentieth aliquot part of a 50.00-cc. sample is employed.

5. Official bismuth preparations are assayed by one of two types of procedure. Illustrate, and calculate the gravimetric factors involved.

6. In view of the insolubility of calcium oxalate, why are official calcium preparations generally not assayed gravimetrically?

7. In the assay of phenobarbital tablets, what change is made in the procedure if stearic acid or stearates are present?

8. Give illustrations of official assay procedures involving (*a*) liberation and filtration of an organic base; (*b*) liberation and extraction of an organic base; (*c*) liberation and filtration of an organic acid; (*d*) liberation and extraction of an organic acid.

9. Give the structural formula and the substance precipitated and weighed in each of the following assay procedures and calculate the gravimetric factor: (*a*) penicillin G potassium; (*b*) phenolphthalein tablets; (*c*) camphor spirit.

10. What weight of tripelennamine dipicrate might be expected in the assay of a 0.1150-Gm. sample of tripelennamine hydrochloride containing 98 per cent $C_{16}H_{21}N_3.HCl$?

Chapter 4

PRINCIPLES OF TITRIMETRIC (VOLUMETRIC) ANALYSIS

In gravimetric analysis, the amount of a reactant, A or B, page 48, present in a sample, is determined by weighing a chemically equivalent amount of a product, C or D, formed from the reactant in a chemical reaction that goes practically to completion. Using the weight of the product and a gravimetric factor, the weight of the reactant in the sample can be calculated. The amount of one reactant, A, can also be determined by measuring the chemically equivalent amount of the other reactant, B, when the two interact in a chemical reaction that goes practically to completion. This is frequently accomplished by adding to the sample containing reactant A a known amount of reactant B in the form of a standard solution, which is equivalent chemically to the reactant A present in the sample. This process, known as *titration*, is common to all *titrimetric* or *volumetric* analyses. In titrimetric or volumetric processes, the *volume* of a standard solution containing a chemically equivalent amount of the reactant B is measured. For example, the amount of NaCl in a sample can be calculated from the weight of a chemically equivalent amount of AgCl obtained in gravimetric analysis or from the volume of a standard solution containing a chemically equivalent amount of $AgNO_3$ employed in titrimetric analysis.

Definitions. A *standard solution* is a solution of known normality or molarity. *Normality* is an expression of the concentration of a solution in terms of equivalents per liter. *Molarity* is an expression of the concentration of a solution in terms of moles per liter. The determination of the normality or molarity of a solution is known as *standardization*. This may be accomplished by use of another standard solution known as a *secondary standard*, or by use of a carefully weighed sample of a substance of known purity which is termed a *primary standard.* Thus a solution of hydrochloric acid may be standardized titrimetrically using a standard solution of sodium hydroxide as a secondary standard or using a sample of sodium carbonate of known purity as a primary standard. Titrations may be conducted *directly*, *e.g.*, by addition of a standard solution of $AgNO_3$ in an amount that is chemically equivalent to the NaCl in a sample. They may also be conducted *residually*, *e.g.*, by addition of a carefully measured volume of standard solution of $AgNO_3$ which is in excess of the amount chemically equivalent to the NaCl in a sample followed by the addition of a standard solution of NH_4SCN in an amount that is chemically equivalent to the excess $AgNO_3$. Obviously, in the residual titration, the known amount of $AgNO_3$ is chemically equivalent to the known amount of NH_4SCN and the unknown amount of NaCl.

In the titration of one reactant with a standard solution of another reactant, the theoretical point at which equivalent amounts of each have reacted is the *stoichiometric point.* The titration is actually stopped at some point, the *end point*, at which there occurs a sudden change in some property of the reaction mixture, a change which is made apparent by use of indicators or potentiometrically. A titration is feasible only when the end point is reasonably close to the theoretical stoichiometric point.

Equivalents of various substances are quantities that are equivalent in a chemical sense, and which may be used to expedite considerably calculations, especially in titrimetric analysis. Whereas 2.016 Gm. of hydrogen are required for reaction with 16 Gm. of oxygen, or 2 moles of hydrogen with 1 mole of oxygen, one equivalent of hydrogen is required for reaction with one equivalent of oxygen. An equivalent of a substance may be expressed in weight units, a quantity known as the *equivalent weight*, or mole fractions. Thus an equivalent of hydrogen is 1.008 Gm. of hydrogen or 0.5 mole of hydrogen. In general, an equivalent is the quantity of a substance that is chemically equivalent to 1.008 Gm. of hydrogen or hydrogen ions. For example, 1 mole or 36.47 Gm. of HCl and 0.5 mole or 49.04 Gm. of H_2SO_4 contain 1.008 Gm. of hydrogen ions; 1 mole or 40.01 Gm. of NaOH and 0.5 mole or 85.69 Gm. of $Ba(OH)_2$

are capable of neutralizing 1.008 Gm. of hydrogen ions; 0.25 mole or 8 Gm. of oxygen gains electrons equivalent to those gained by 1.008 Gm. of hydrogen ions when they act as oxidizing agent, and 1 mole or 166.02 Gm. of KI loses electrons equivalent to those lost by 1.008 Gm. of hydrogen when it acts as reducing agent.

A *milliequivalent* is one-thousandth of an equivalent and, for HCl, may be variously expressed as 0.001 mole, 0.001 equivalent, 0.03647 Gm., or 1 cc. of $1N$ solution.

Volumetric Apparatus. The apparatus required for volumetric analysis includes the analytical balance described in Chapter 1 and a number of accurately calibrated instruments, as well as beakers, flasks, funnels, etc. Volumetric apparatus is of two types: that made to deliver a definite volume of liquid, as burettes and pipettes; and that made to contain a definite volume of liquid, as volumetric flasks and graduated cylinders. The temperature specified in the U.S.P. for volumetric measurements is 25°, while the National Bureau of Standards has adopted 20° as the standard temperature for glass volumetric apparatus. It matters little at what temperature the instruments are calibrated, provided the same temperature is used for all, and all measurements are made at the temperature for which the instruments are calibrated.

Units of Capacity. The *cubic centimeter* (cc.) represents the one-thousandth part of the liter. The *liter* is defined as the volume occupied by a kilogram of water weighed in vacuum at 4°. The one-thousandth part of a liter, termed a *milliliter*, and the *cubic centimeter* are both used as the unit of capacity. The cubic centimeter, abbreviated as "cc.," which has been adopted as the official unit of capacity, is not exactly the one-thousandth part of a liter, since one milliliter = 1.000027 cc. The difference is so slight, however, that it becomes negligible in most volumetric measurements.

Burettes. Graduated glass tubes of uniform bore throughout the whole length, used in the measurement of variable quantities of liquid, are termed *burettes* (Figs. 9 and 10). They are graduated into cubic centimeters and closed at the bottom by a glass stopcock or a short, soft rubber tube provided with a glass tip and pinchcock or other suitable device to control the outflow of liquid. The official requirements for burettes follow:

All burettes should be provided with glass stopcocks and calibrated for use at the standard or other temperature at which they are to be used. Burettes provided with rubber tubes, pinch-cocks, and glass delivery tubes in place of glass stopcocks may be used for potassium and sodium hydroxide solutions and such other solutions as are best used in this type of burette. The rate of outflow should be regulated so that not less than 2 seconds should be consumed for each cubic centimeter delivered.

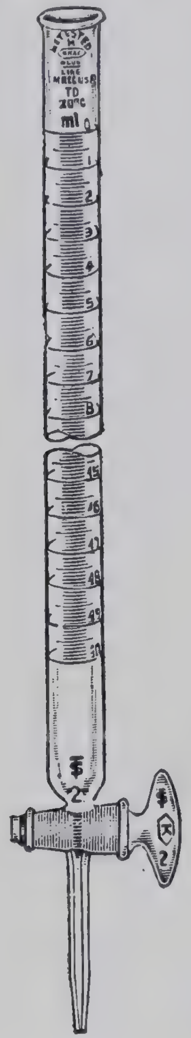


FIG. 9. Burette with glass stopcock.

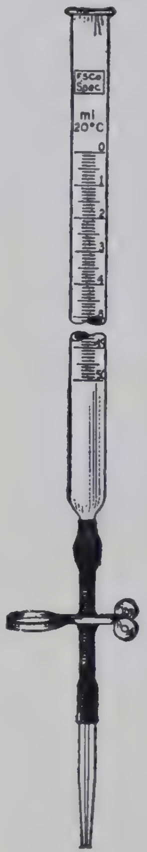


FIG. 10. Burette with pinchcock. (Mohr burette.)

Burettes provided with glass stopcocks should be tested for leakage. A lubricant serves to prevent leakage and permits easy operation, but an excess of lubricant should never be used. Those provided with a rubber outlet (Mohr burettes) should never be used for other than alkali solutions. The outlet tip of either type of burette should be of such diameter and taper as to permit the delivery of a drop the volume of which is considerably less than that which can be accommodated between the finest graduations of the scale with which the burette is marked.

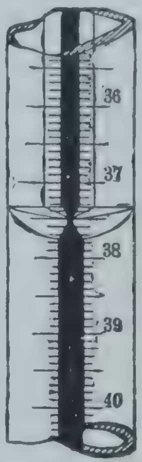


FIG. 11. Effect of parallax in reading burettes.

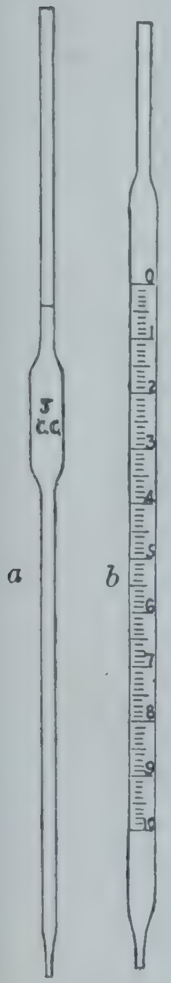


FIG. 12. (*a*) Transfer or delivery pipette. (*b*) Measuring pipette.

Reading Burettes. The surface of liquids in narrow tubes is always curved due to capillarity; the surface or *meniscus* is always concave when the liquid wets the tube and convex when it does not. Readings should always be made at the lowest point of the meniscus, *i.e.*, the graduation mark that coincides with the bottom of the curve, except when measuring highly colored liquids. The eye must be on the same level as the meniscus; otherwise error due to parallax will be introduced (see Fig. 11). Numerous devices have been employed to assist in reading burettes correctly. One such device recommended by the National Bureau of Standards is a collar-shaped section of black rubber tubing, cut open at one side and of a size that fits the burette tube so that it can be moved. This black rubber tubing, when placed immediately below the meniscus, renders the meniscus profile dark and clearly observable against a light background.

Pipettes. The following requirements for transfer pipettes have been adopted in the U.S.P.:

> Pipettes should be graduated to deliver at standard temperature the volume indicated. Pipettes should never be drained by blowing into them unless they are especially graduated for use in this way.

The limits of error permitted in the calibration of pipettes shall be those accepted by the United States Bureau of Standards, which are as follows:

Contents of pipettes	2 cc.	5 cc.	10 cc.	25 cc.	50 cc.	100 cc.
Limit of error in cc	0.006	0.01	0.02	0.03	0.05	0.08
Limit of error in per cent	0.30	0.20	0.20	0.12	0.10	0.08

It is essential that in using pipettes the same procedure be employed as was used in the calibration.

When transfer or delivery pipettes (Fig. 12) are used, they are filled to about 1 cm. above the mark. Any liquid adhering to the outer surface is carefully wiped off and the pipette is allowed to drain down just to the mark. The liquid is drawn up by sucking the upper end of the pipette with the mouth, but this procedure should be avoided and specially designed pipettes or some other method of measurement employed when volatile, corrosive, or poisonous liquids are measured.

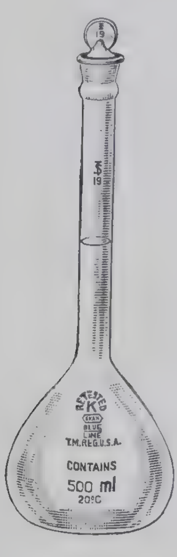


FIG. 13. Volumetric flask.

Measuring Flasks. The U.S.P. requirements for measuring or volumetric flasks (Fig. 13) are as follows:

Standard measuring flasks are calibrated to contain the indicated volume, at 25°, when filled to the mark.

The limits of error permitted in the calibration of flasks shall be those accepted by the United States Bureau of Standards, which are as follows:

Contents of flask	50 cc.	100 cc.	250 cc.	500 cc.	1000 cc.
Limit of error in cc	0.05	0.08	0.12	0.15	0.30
Limit of error in per cent	0.10	0.08	0.05	0.03	0.03

Volumetric flasks are used to make up standard solutions to a given volume. Note that the U.S.P. requires that flasks be graduated to contain, and not to deliver, the calibrated volume.

Graduated Cylinders. Graduated cylinders are used in making approximate measures of volume. They are graduated to contain a given volume of liquid at standard temperature. The inside diameter of the cylinder should not exceed one-fifth of the graduated length.

Cleaning Volumetric Apparatus. New volumetric apparatus should always be cleaned before using, and that in use should be cleaned as often as necessary. The adherence of droplets to the wall of a burette or pipette is positive evidence that the apparatus is dirty. In a clean instrument, the liquid drains down uniformly, wetting the walls so that no droplets are observable. A warm cleaning solution (sodium dichromate in sulfuric acid), solution of sodium hydroxide, or solution of soap powder is the best cleansing agent to use. Hot solutions should be avoided when cleaning accurately calibrated apparatus because of the possible production of a permanent change in volume caused by the heat and known as *thermal aftereffect.*

Calibration of Volumetric Apparatus. The ordinary volumetric apparatus marketed is calibrated at various temperatures and is often unreliable. The analyst should always check the calibration of volumetric apparatus and make corrections if necessary.

Burettes. The calibration of burettes may be performed in various ways by means of special calibration devices, such as the Ostwald calibrating pipette and the Kiehl burette calibrator. In all practical work, however, the following method is satisfactory:

The clean burette, previously tested for leakage, is filled to the zero mark with distilled water. The burette and water used should be in the room a sufficient length of time before use to acquire room temperature. A definite volume, *e.g.*, 10 cc., is allowed to run into a tared weighing bottle. The weight of water withdrawn is determined, and the burette is filled again to the zero mark, and then 20 cc. is withdrawn in exactly the same way. This operation is repeated for each 10 cc. until the 50-cc. mark is reached, determining the weight of water withdrawn each time. The results with corrections should be recorded in tabular form showing the relation between the apparent volume and actual weight delivered to each mark on the burette corresponding to an interval of 10 cc. Weighings need not be more accurate than to the second decimal place, since that is the greatest degree of accuracy with which the burette can be read.

A burette calibrated at 20° should deliver 9.9718 Gm. of water, or at 25° it should deliver 9.9604 Gm. for each 10 cc., since 1 liter of distilled water at 20° weighs 997.177 Gm. and at 25°, 996.04 Gm. when weighed with brass weights in air of 50 per cent humidity at standard atmospheric pressure. The true volume for each 10-cc. segment of the burette may be calculated from the weights obtained and recorded on a convenient chart.

Pipettes. Clean the pipette thoroughly with soap solution and cleaning mixture and rinse well with distilled water. Fill it with distilled water at 20 or 25°, according to the temperature selected for calibration, and allow it to drain into a tared weighing bottle. Weigh accurately and record the correct volume of the pipette.

Flasks. Clean, dry, and tare the flask, placing the number of grams weight corresponding to its volume on the opposite balance pan and introduce water at 20 or 25° into the flask in sufficient quantity to counterbalance the added weights. Mark the lowest point of the meniscus if it does not agree with the original calibration.

Sources of Error in the Use of Volumetric Apparatus. Every precaution should be taken to avoid the following common sources of error in the use of volumetric apparatus:

1. Rinse water adhering to walls of apparatus. The apparatus may be dried, but it is more convenient and just as accurate to wash it out with small successive portions of the liquid to be used, discarding the washings.

2. Grease films and dirty apparatus cause irregularity in the delivery of liquids and distort the meniscus.

3. Drainage or afterflow of the liquid adhering to the vessel walls to the liquid below necessitates that the time of drainage be the same as that used in the calibration of the instrument.

4. Parallax must be avoided to secure proper readings of the level of the meniscus.

5. Variations in temperature lead to changes in volume of vessels and liquids. All measurements, therefore, should be made at a temperature closely approximating that at which the apparatus was calibrated.

6. Air bubbles trapped beneath the liquid surface, especially below the stopcock in burettes, displace liquid.

7. Heat, such as supplied by hot solutions, causes calibrated volumetric apparatus to suffer a slight permanent change of volume.

8. Most salts when dissolved produce a change in temperature, with concomitant change in volume of the solution. Such solutions should never be measured until they have acquired the temperature at which the instruments used to measure them were calibrated.

9. Failure to use apparatus in a manner approaching as nearly as possible that followed in calibration.

Questions and Problems

1. Define the following terms: (*a*) *standardization;* (*b*) *standard solution;* (*c*) *end point;* (*d*) *residual titration;* (*e*) *primary standard;* (*f*) *equivalent;* (*g*) *equivalent weight.*

2. What is the difference between a cubic centimeter and a milliliter?

3. Burettes provided with rubber tubes and pinchcocks in place of glass stopcocks may be used to measure out sodium or potassium hydroxide solution, but they should never be used in the measurement of solutions of acids or oxidizing agents. Why?

4. A 100-cc. volumetric flask calibrated at 25° is used to measure water at 20°. What correction of the volume must be made? (See U.S.P.)

5. A volumetric flask calibrated to contain 100 cc. at 25° and 760 mm. barometric pressure is used at 18° and 746 mm. barometric pressure. Calculate the volume of water contained in the flask at the temperature and pressure at which it is used.

NEUTRALIZATION METHODS—ACIDIMETRY AND ALKALIMETRY

Acidimetry is the measurement of the quantity of acid in a given sample by titration with a suitable alkali. *Alkalimetry* is the measurement of the quantity of alkali or base in a given sample by titration with a suitable acid. In each case the principle involved is the same, *viz.*, the addition of a chemically equivalent amount of a standard solution of a base or acid to the sample of acid or base, respectively, being assayed. From the volume or the known number of equivalents of standard solution added, the weight, an equal number of equivalents of acid or base present in the sample can be readily calculated. The equivalent weight of the acid or base being assayed can be obtained by examination of the formula of the acid or base or the balanced equation for the reaction involved.

Before undertaking analyses by methods of acidimetry and alkalimetry, the student should become familiar with the theoretical considerations involved.

Titer. Interpretation of the data obtained in titrimetric analysis is facilitated considerably by use of a chemical factor known as the *titer* of a solution, corresponding to the *gravimetric factor* used in gravimetric analysis. *Titer* may be defined as the weight of solute per cubic centimeter of a solution or, more commonly, as the weight of a substance chemically equivalent to 1 cc. of a standard solution. Thus 1 cc. of 1*N* hydrochloric acid contains 0.03647 Gm. (0.001 equivalent or 1 milliequivalent) of HCl and is, therefore, equivalent chemically to 0.04001 Gm. (0.001 equivalent or 1 milliequivalent) of NaOH.

Titers of standard solutions used in official assay procedures are specified in the various monographs. For example, in the U.S.P. procedure for the assay of calcium hydroxide solution for $Ca(OH)_2$, it is stated: "Each cubic centimeter of 0.1*N* hydrochloric acid is equivalent to 3.705 mg. of $Ca(OH)_2$." The $Ca(OH)_2$ titer of 0.1*N* hydrochloric acid is, therefore, 3.705 mg. per cc., a value that can be calculated as follows: Examination of the formula, $Ca(OH)_2$, or of the equation,

$$\underset{74.10}{Ca(OH)_2} + 2HCl \longrightarrow CaCl_2 + 2H_2O$$

indicates that 1 mole or 74.10 Gm. of $Ca(OH)_2$ is 2 equivalents, and the equivalent weight of $Ca(OH)_2$ is 37.05 Gm. Each cc. of 0.1*N* hydrochloric acid contains 0.0001 equivalent of HCl and is equivalent to 0.0001 equivalent or $0.0001 \times 37.05 = 0.003705$ Gm. or 3.705 mg. of $Ca(OH)_2$.

Theory. For the purposes of acidimetry and alkalimetry, an *acid* may be defined as an ionic or molecular substance capable of giving up a proton, *i.e.*, a proton donor. A *base* may be defined as an ionic or molecular substance capable of uniting with a proton, *i.e.*, a proton acceptor. For example, H_2O, H_2S, HCl, and H_2SO_4 are molecular acids; NH_3 and H_2O are molecular bases; H_3O^+ and NH_4^+ are cationic acids; $Ag(NH_3)_2^+$ is a cationic base; HSO_4^-, $H_2PO_4^-$, $HPO_4^=$, and HS^- are anionic acids; and OH^-, SH^-, $S^=$, and $SO_4^=$ are anionic bases.

In the water system,

$$2H_2O \rightleftharpoons H_3O^+ + OH^-$$

acid properties, such as taste, effect on indicators, and oxidation of metals high in the electromotive series, depend on the presence of the hydronium ion, H_3O^+. It is generally written as a hydrogen ion, H^+, for convenience. Substances which, on dissolving in water, increase the concentration of the hydronium ion also increase the acid properties of the solution, and are, therefore, acids.

$$HCl + H_2O \longrightarrow H_3O^+ + Cl^-$$
$$NH_4^+ + H_2O \rightleftharpoons H_3O^+ + NH_3$$
$$HSO_4^- + H_2O \rightleftharpoons H_3O^+ + SO_4^=$$

Monoprotic acids are those which are capable of giving up 1 proton per molecule or ion; diprotic, 2 protons, etc. The tendency of an acid to give up a proton is the principal determinant of the strength of the acid. Many of the mineral acids appear to have equal strengths in aqueous solution because water is a sufficiently strong base for the following reaction to go practically to completion:

$$HA + H_2O \rightleftharpoons H_3O^+ + A^-$$

In such cases, water is said to have a leveling effect. When weaker acids are dissolved in water, the above reaction is reversible.

Proton acceptors, or bases, when dissolved in water, reduce the concentration of hydronium ions as illustrated by the following:

$$OH^- + H_3O^+ \rightleftharpoons 2H_2O$$
$$NH_3 + H_3O^+ \rightleftharpoons NH_4^+ + H_2O$$
$$SO_4^= + H_3O^+ \rightleftharpoons HSO_4^- + H_2O$$

Those bases which can accept one proton are monoprotic; those which can accept two protons are diprotic, etc. The stronger a base is, the greater is its tendency to accept protons.

Since a water molecule may donate or accept a proton, it is both an acid and a base. Thus, two water molecules may interact to form ions: $H_2O + H_2O \rightleftharpoons H_3O^+ + OH^-$. The process is called *autoprotolysis*. When there is simply a transfer of a proton from one molecule to another, the process is called *protolysis*, or is referred to as a *protolytic reaction*. An acid and a base related to each other so that one is formed from the other by the gain or loss of a proton are said to be corresponding or *conjugate* to one another. Thus Cl^-

is the *conjugate base* of HCl, CH_3COO^- is the conjugate base of CH_3COOH, and NH_3 is the conjugate base of the acid NH_4^+. Similarly, HCl is the conjugate acid of the Cl^-, CH_3COOH is the conjugate acid of the CH_3COO^-, and NH_4^+ is the conjugate acid of NH_3. A proton does not tend to leave an acid unless a base is present to accept the proton. The fundamental acid-base relationships may be expressed as follows:

$$\text{Acid} \rightleftharpoons H^+ \text{ (proton)} + \text{base}$$

The transfer of a proton from an acid results in the formation of a new acid and a new base:

$$\begin{gathered}
\text{Acid} + \text{base} \rightleftharpoons \text{acid}' + \text{base}' \\
HCl + H_2O \longrightarrow H_3O^+ + Cl^- \\
CH_3COOH + OH^- \rightleftharpoons H_2O + CH_3COO^- \\
H_2O + NH_3 \rightleftharpoons NH_4^+ + OH^-
\end{gathered}$$

The weakly basic chloride ion loses its proton to the more strongly basic water molecule. The acetate ion, being a stronger base than the water molecule, retains most of its protons. The ammonia accepts a proton from the water molecule to form the acidic ammonium ion.

For the purpose of brevity, the term *hydrogen ion* and the symbol H^+ are used in this book for the hydronium ion.

The hydrogen ion concentration influences most reactions employed in analytical methods, whether they are neutralization, precipitation, or oxidation-reduction reactions.

It has been established that in any aqueous solution there are always hydrogen and hydroxide ions present and that, whatever the concentration of the one, the concentration of the other is such that the product of their concentrations is a constant for any given temperature.

$[H^+] \times [OH^-]$ = a constant for any given temperature, and in pure water at about 22°, $[H^+] \times [OH^-] = 1 \times 10^{-14}$. Since this relationship is true, the concentration of hydrogen ions $[H^+]$ expressed in terms of gram-ions per liter in pure water is equal to the square root of $1 \times 10^{-14} = 1 \times 10^{-7}$ (0.0000001), and the hydroxyl ion concentration $[OH^-]$ also is equal to 1×10^{-7}. The degree of acidity or alkalinity of a solution can, therefore, be defined in terms of its concentration of hydrogen ion or hydroxide ion, since when the value of one is known, the value of the other can be calculated: *i.e.*, if $[H^+] = 10^{-5}$, then $[OH^-] = 10^{-9}$, since $[H^+] \times [OH^-]$ must equal 10^{-14}. Solutions which have a hydrogen ion concentration greater than 10^{-7} are acidic, and those which have a hydrogen ion concentration less than 10^{-7} are alkaline. Thus a solution of 0.01*N* HCl contains 10^{-2} Gm.-ion of hydrogen per liter, assuming complete dissociation, and is acid in reaction although it contains 10^{-12} Gm.-ion of OH^-.

To avoid the disadvantage of employing a dual system of definition, it is customary to use only the hydrogen ion concentration to characterize a solution, whether it be acidic, neutral, or alkaline, the hydroxide ion concentration being implied.

pH Value. The reaction, acidity, alkalinity, or neutrality of a solution may be defined not only in terms of the molecular concentrations of hydrogen ions, numbers frequently so small as to be quite cumbersome, but also in pH values, a system suggested by Sörensen and based on the relationship between the electromotive force and hydrogen ion concentration of certain cells. An important factor in this relationship is the logarithm of the reciprocal of the hydrogen ion concentration (page 433). This factor has become known as the pH value or simply as pH.

pH is defined as the logarithm of the reciprocal of the hydrogen ion concentration, and is expressed as

$$\text{pH} = \log \frac{1}{[H^+]}$$

Since the log of a quotient equals the difference between the log of the dividend and the log of the divisor,

$$\begin{aligned} \text{pH} &= \log(1) - \log([H^+]) \\ &= 0 - \log([H^+]) \\ &= -\log([H^+]) \end{aligned}$$

pH values can be calculated from hydrogen ion concentrations as in the following examples:

$$
\begin{aligned}
(a)\ [H^+] &= 0.0001 = 10^{-4} \text{ Gm.-ion per liter} \\
pH &= -\log\,([H^+]) \\
pH &= -\log\,(10^{-4}) \\
&= -(-4) \\
&= 4 \\
(b)\ [H^+] &= 2.73 \times 10^{-4} \text{ Gm.-ion per liter} \\
pH &= -\log\,(2.73 \times 10^{-4}) \\
&= -[\log\,(2.73) + \log\,(10^{-4})] \\
&= -\log\,(2.73) - \log\,(10^{-4}) \\
&= -0.4362 + 4 \\
&= 3.5638 \text{ or } 3.564
\end{aligned}
$$

Conversely, when the pH value is known, the hydrogen ion concentration can be calculated as in the following examples:

(*a*)

$$
\begin{aligned}
pH &= 4 \\
-\log\,[H^+] &= 4 \\
\log\ H^+] &= -4 \\
[H^+] &= 10^{-4}
\end{aligned}
$$

(*b*)

$$
\begin{aligned}
pH &= 3.56 \\
-\log\,[H^+] &= 3.56 \\
\log\,[H^+] &= -3.56 \\
&= -4 + 0.44 \\
[H^+] &= 10^{-4} \times 2.75
\end{aligned}
$$

Two practical methods for determining when the hydrogen ion concentration in a solution has acquired a certain value have been developed, *viz.*, the potentiometric method and the colorimetric method (page 434).

Buffers. In many analytical procedures, a change in the hydrogen ion concentration occurs as a result of chemical reaction. In certain cases, this change in the concentration of hydrogen ion may prevent the reaction from going to completion quantitatively. Since the change in hydrogen ion concentration is caused by the formation of acid or base in the course of the reaction, some provision must be made to eliminate the acid or base formed in such a manner as to maintain a sensibly constant hydrogen ion concentration. This is usually accomplished by means of a buffer. The term buffer is applied alike to the salts of weak acids and to the salts of weak bases. Solutions of these salts resist change in hydrogen ion concentration on the addition of small amounts of either acid or base. In the titration of acetic acid with sodium hydroxide, the sodium acetate formed acts as a buffer to prevent a large change in hydrogen ion concentration. Acetic acid in aqueous solution is slightly dissociated as shown in the following equations:

$$HC_2H_3O_2 \rightleftharpoons H^+ + C_2H_3O_2^- \qquad (1)$$

$$Na^+OH^- + H^+ \longrightarrow Na^+ + H_2O \qquad (2)$$

$$HC_2H_3O_2 + Na^+OH^- \rightleftharpoons Na^+C_2H_3O_2^- + H_2O \qquad (1) + (2)$$

In reaction (1), the slightly dissociated acetic acid forms H^+ and $C_2H_3O_2^-$ which combine with OH^- and Na^+, respectively, to form water and sodium acetate [Eq. (2)]. In the equilibrium mixture, both $HC_2H_3O_2$ and $C_2H_3O_2^-$ are present. If $[H^+]$ is reduced by addition of more NaOH or other base, the $HC_2H_3O_2$ dissociates as in Eq. (1) and thus prevents a lasting change in $[H^+]$. On the other hand, if an acid such as HCl is added to the mixture, $[H^+]$ does not increase greatly because of the combination of H^+ with $C_2H_3O_2^-$ [reverse of reaction (1)]. This buffering action, preventing any great change in $[H^+]$, will continue on further addition of acid or base as long as $HC_2H_3O_2$ or $Na^+C_2H_3O_2^-$ are present.

The principle of buffer action is often employed in analytical methods in which acid or base is generated as a result of the analytical reaction. In such cases, the addition of an amount of buffer salt relatively large with respect to the amount of acid or base generated results in a fractional diminution of the buffer ion through its union with the hydrogen ion to form the slightly dissociated acid corresponding to the buffer salt employed. The diminution in the amount of buffer salt will then be so slight that the hydrogen ion concentration will remain practically constant. In iodimetric assays where HI is formed as a product of the reaction, sodium bicarbonate is used as a buffer to maintain an approximately constant hydrogen ion concentration.

Neutralization Reactions. It is important to remember that, in terms of the theory of ionization, a neutral solution contains H^+ and OH^- in the same concentration as in pure water, *viz.*, 0.0000001 Gm.-ion per liter of solution. Any substance which can furnish excess H^+ when dissolved in water is acidic and any substance which can furnish excess OH^- when dissolved in water is basic. From the point of view of the theory of ionization, therefore, *neutralization* is the union of H^+ from an acid with OH^- from a base, to form molecules of water. The other ions furnished by the acid or base may or may not combine.

Neutralization reactions must proceed very nearly to completion to be of value in quantitative analysis. Reactions may be caused to go to completion in a number of ways, *i.e.*, by the formation of a slightly dissociated substance as a reaction product; by the removal of one or more of the products of the reaction as a gas; by the removal of one or more of the products of reaction as a precipitate; by the removal of an ionic substance as a complex ion; by changing the charge of an ion; and by adding an excess of one of the reactants.

Solutions of HCl and NaOH in water are dispersions of ions (100 per cent ionized), since each of these substances is a strong electrolyte. When two such solutions are mixed, the hydrogen ions of the HCl solution combine with the hydroxide ions of the NaOH solution, forming the very slightly ionized water molecules and leaving an equivalent amount of sodium ions and chloride ions dispersed in the solution as sodium chloride.

$$H^+Cl^- + Na^+OH^- \longrightarrow Na^+Cl^- + H_2O \qquad (1)$$

The neutralization reaction indicated by Eq. (1) goes to completion. In titrating a solution of HCl with a solution of NaOH, an excess of hydrogen ions will be present because of the HCl until an equivalent amount of NaOH has been added. If the stoichiometric point and the end point coincide, hydrogen ions would be present only as a result of dissociation of water molecules and

$$[H^+] = [OH^-] = 1 \times 10^{-7} \qquad \text{or} \qquad pH = 7$$

It is not practical, however, in this titration, to avoid addition of a very slight excess of NaOH, with the result that the pH at the end point is generally considerably higher than 7.

Admixture of solutions of acetic acid, $HC_2H_3O_2$, and NaOH results in an equilibrium mixture at the stoichiometric point in which are present the substances formed by neutralization [Eq. (2)], *viz.*, $Na^+C_2H_3O_2^-$ and the very slightly ionized H_2O; and those formed by the reverse reaction [hydrolysis, Eq. (3)], *viz.*, Na^+OH^- and slightly ionized $HC_2H_3O_2$.

$$Na^+OH^- + HC_2H_3O_2 \underset{(3)}{\overset{(2)}{\rightleftharpoons}} Na^+C_2H_3O_2^- + H_2O$$

This reaction does not go to completion, and there is an excess of OH^- ions at the stoichiometric point when acetic acid is titrated with sodium hydroxide. Such a reversible reaction, however, may be used as the basis for a quantitative analytical procedure, provided that the end point of the titration can be brought close to the stoichiometric point, *e.g.*, by proper choice of indicator.

Indicators. The indicators commonly used to determine the end point in neutralization processes are, as a rule, complex organic compounds capable of existing in two forms of different color that are mutually convertible, one into the other, at given $[H^+]$ or pH. To explain the change in color of indicators, three theories have been proposed which, briefly stated, are as follows:

1. The physicochemical theory attributes the color to certain ions an increase of which causes the appearance of a new color, and the decrease of which causes the disappearance of a color or the appearance of a different color.

2. The organic theory attributes the color of indicators to certain groupings of the elements in a compound, and the change in color to change in molecular structure.

3. The colloidal theory assumes that indicators form colloidal solutions the change in color of which is dependent upon change in size of the colloidal particle.

The point at which an indicator changes color in any given titration is dependent on the hydrogen ion concentration of the solution and may not be indicative of the absolute neutrality or completion of a reaction. The accompanying table of the commonly used indicators gives the $[H^+]$ expressed in terms of pH between which color changes occur.

This table shows that methyl orange exhibits its acid color, pink, at a pH of 3.1 and its alkaline color, yellow, at a pH of 4.4, while between these pH values the color undergoes transition from one shade to the other. At a pH of 4.4 the solution is slightly acid, since at neutrality pH = 7.

TABLE 13. COMMONLY USED INDICATORS

Indicator	pH range	Color	
		Acid	Alkaline
Bromocresol green	3.8 to 5.4	Yellow	Blue
Bromocresol purple	5.2 to 6.8	Yellow	Purple
Bromophenol blue	3.0 to 4.6	Yellow	Blue
Bromothymol blue	6.0 to 7.6	Yellow	Blue
Litmus	4.5 to 8.0	Red	Blue
Methyl orange	3.1 to 4.4	Pink	Yellow
Methyl red	4.2 to 6.3	Red	Yellow
Methyl yellow	2.9 to 4.0	Red	Yellow
Phenol red	6.8 to 8.4	Yellow	Red
Phenolphthalein	8.3 to 10.0	Colorless	Red
Thymol blue	1.2 to 2.8	Red	Yellow
	8.0 to 9.6	Yellow	Blue
Thymolphthalein	9.3 to 10.5	Colorless	Blue

It would appear that an indicator which changes color exactly at the neutral point, pH = 7, would be required in every case, but this is not true. When a strong acid, such as hydrochloric acid, is titrated with a strong base, like sodium hydroxide, the change in concentration of hydrogen ion becomes very rapid as the equivalence point is approached, so that very small amounts of acid or alkali in excess carry the concentration of hydrogen ion far to one side of the neutral point, and the indicator will give a sharp end point. If a solution of 25 cc. of $0.1N$ HCl is titrated with a solution of $0.1N$ NaOH, the equivalent point would be reached when 25 cc. of $0.1N$ NaOH had been added. Theoretically, the solution at the end of titration should be identical in composition with a solution prepared by dissolving a known weight of sodium chloride in the calculated amount of solvent. When methyl orange is used as indicator, the end point, as shown by change of color, appears when about 24.95 cc. of $0.1N$ NaOH have been added or while the solution is still slightly acidic, but if phenolphthalein is used as indicator, the end point appears when about 25.05 cc. of $0.1N$ NaOH have been added or when the solution is slightly alkaline. The deviation of the end point from the equivalence point is so slight that it is negligible in this case.

When a weak acid is titrated with a strong base, the change in hydrogen ion concentration in the vicinity of the equivalence point is not so abrupt; *e.g.*, if 25 cc. of 0.1*N* acetic acid are titrated with 0.1*N* NaOH, 25 cc. of the alkali should be required to attain the equivalence point. When methyl orange is employed as indicator, the end point is reached when about 17.5 cc of 0.1*N* NaOH have been added, but if phenolphthalein is used, the end point is reached when practically 25 cc. of 0.1*N* NaOH have been added. Obviously methyl orange would not be a satisfactory indicator in this titration.

If 0.1 cc. of 0.1*N* HCl is added to a liter of 0.1*N* NaCl, the $[H^+]$ changes from 10^{-7}, the concentration in dilute solutions of neutral salts, to 10^{-4}, the concentration in a 0.0001*N* HCl solution. If 0.1 cc. of 0.1*N* HCl or NaOH is added to a liter of 0.1*N* sodium acetate, no appreciable change in $[H^+]$ takes place. Likewise, in the titration of acetic acid with sodium hydroxide, the acetic acid-sodium acetate acts as a buffer solution to prevent large changes in $[H^+]$. The acetate ions from the highly ionized sodium acetate force the reaction to the left $CH_3COOH \rightleftharpoons H^+ + CH_3COO^-$ before an equivalent quantity of NaOH has been added, and the hydrogen ion concentration becomes very small before all the hydrogen of acetic acid has been replaced. Methyl orange, which changes color at a relatively high hydrogen ion concentration, $10^{-4.4}$, will gradually show alkaline color long before the equivalent quantity of alkali has been added, and the end point will not be sharp. Phenolphthalein, however, does not change color until the true neutral point $pH = 7$ has been passed, and a drop of alkali will then reduce the concentration below $[H^+] = 10^{-9.8}$ or $pH = 9.8$ where phenolphthalein exhibits a change of color.

A weak base titrated by a strong acid, *e.g.*, ammonium hydroxide titrated with sulfuric or hydrochloric acid, exhibits the reverse of the above condition, as would be expected, and methyl red is a suitable indicator whereas phenolphthalein is not.

Rules for the Use of Indicators. The following rules should be observed in the use of indicators:

1. Use two drops of indicator solution for a titration unless otherwise directed.

2. When a strong acid is titrated with a strong alkali, or a strong alkali with a strong acid, then methyl orange, methyl red, or phenolphthalein may be used.

3. When a weak acid is titrated with a strong alkali, use phenolphthalein as indicator.

4. When a weak alkali is titrated with a strong acid, use methyl red as indicator.

5. A weak alkali should never be titrated with a weak acid or vice versa, since no indicator will give a sharp end point.

6. The appearance of a color is more easily observable than is the disappearance. Always titrate where possible, therefore, to the appearance of a color.

The criteria of a good indicator for analytical purposes are that there shall be a sharp contrast between the two colors which it exhibits in acid and alkaline mediums and that the change in color shall take place over a very small range of pH values.

Some Commonly Used Indicator Solutions.

Bromocresol Green T.S. A solution of 50 mg. of bromocresol green, tetrabromo-*m*-cresolsulfonphthalein, in 100 cc. of alcohol, filtered if necessary.

Bromocresol Purple T.S. A solution of 50 mg. of bromocresol purple, dibromo-*o*-cresolsulfonphthalein, in 100 cc. of alcohol, filtered if necessary. Two to four drops of the indicator solution per 100 cc. should be used in titrations unless otherwise directed.

Bromophenol Blue T.S. A solution of 100 mg. of bromophenol blue, tetrabromophenolsulfonphthalein, in 100 cc. of diluted alcohol, filtered if necessary. Four to six drops of the indicator solution per 100 cc. should be used in titrations.

Bromothymol Blue T.S. A solution of 100 mg. of bromothymol blue, dibromothymolsulfonphthalein, in 100 cc. of diluted alcohol, filtered if necessary. Two to four drops of indicator solution per 100 cc. should be used in titrations.

Litmus T.S. A solution prepared by extracting for 1 hr. 25 Gm. of powdered litmus with three 100-cc. portions of boiling alcohol, filtering and washing the undissolved residue with alcohol, macerating the residue with 25 cc. of cold water for 1 hr., filtering and finally digesting the residue with 125 cc. of boiling water for 1 hr., cooling and filtering. Litmus, or tournesol, consists of a blue pigment, chiefly azolitmin, obtained from various species of Roccella (De Candolle) by fermentation. The pigment mixed with chalk or other diluent is marketed in cubes or fragments.

Other indicator solutions are superior to litmus for titration purposes, but the test solution serves well for rough qualitative tests of hydrogen ion concentration.

Methyl Orange T.S. A solution of 100 mg. of methyl orange, sodium dimethylaminoazobenzenesulfonate, in 100 cc. of water, filtered if necessary. Two drops of the indicator solution per 100 cc. should be used in titrations.

The color change is very sharp unless too much indicator is used. Methyl orange is used frequently in the titration of strong acids and strong alkalies. It is also a good indicator to use in the titration of the salts of weak acids, such as carbonates, borates, sulfides, etc., with a strong acid, since in these cases the acids liberated in the titration reaction are too little ionized to affect the indicator. It does not give the proper end point in the titration of alkaloids or organic acids. This indicator should never be employed in the titration of alcoholic solutions, hot solutions, or very dilute solutions.

Methyl Red T.S. A solution of 100 mg. of methyl red, paradimethylaminoazobenzene-ortho-carboxylic acid, in 100 cc. of alcohol, filtered if necessary. Two drops of the indicator solution per 100 cc. should be used in titrations. Methyl red T.S. is especially useful in titrating solutions of ammonia, other weak bases and alkaloids, but it is not suitable for the titration of weak organic acids.

Phenol Red T.S. A solution of 100 mg. of phenol red, phenolsulfonphthalein, in 100 cc. of alcohol, filtered if necessary.

Phenolphthalein T.S. A solution of 1 Gm. of phenolphthalein in 100 cc. of alcohol. Two to three drops of the indicator solution per 100 cc. are used in titrations unless otherwise directed. Phenolphthalein is an excellent indicator to use in titration of weak acids, most alkalies, and alkali salts; but is not satisfactory for titration of ammonia, alkaloids, or cold solutions of carbonates and bicarbonates. It functions well in alcoholic solutions.

Thymol Blue T.S. A solution of 100 mg. of thymol blue, thymolsulfonphthalein, in 100 cc. of alcohol, filtered if necessary.

Thymolphthalein T.S. A solution of 100 mg. of thymolphthalein in 100 cc. of alcohol, filtered if necessary.

Sensitivity of Indicator Solutions. Indicator solutions should be tested for sensitivity. This is done by adding 0.15 cc. of indicator solution to 25 cc. of carbon dioxide–free distilled water and titrating the resulting solution with $0.02N$ alkali or acid. Upon the addition of 0.25 cc. of the standard alkali or acid solution, the respective characteristic color should develop.

Questions and Problems

1. Define the following terms: acidimetry, titer, milliequivalent, pH, and buffer.

2. Calculate the pH of each of the following solutions: (*a*) $0.025M$ hydrochloric acid; (*b*) $0.01M$ sodium hydroxide; (*c*) $0.1M$ acetic acid, $K_i(HC_2H_3O_2) = 1.8 \times 10^{-5}$; (*d*) 2 per cent sodium acetate W/V.

3. Calculate the hydrogen ion concentration of a solution with a pH of 5.69, and the hydroxide ion concentration of a solution with a pH of 8.31.

4. What indicator is best suited for use in the following titrations? Give reasons. (*a*) Alkali carbonate with sulfuric acid; (*b*) acetic acid with sodium hydroxide; (*c*) ammonia solution with hydrochloric acid; (*d*) organic acids with sodium hydroxide.

5. How may the sensitivity of an indicator solution be determined?

Standard Solutions. The concentrations of standard solutions can be expressed in terms of *normality*, or the number of equivalents of solute per liter of solution, or in terms of *molarity*, or the number of moles of solute per liter of solution. In certain types of calculation, the *molality*, or the number of moles of solute per 1,000 Gm. of solvent, is used.

A tenth-normal solution of hydrochloric acid, also designated as $0.1N$ or $N/10$, contains 0.1 equivalent or 0.1 equivalent weight, 3.647 Gm., of HCl per liter, or 0.1 milliequivalent, 3.647 mg., per cc. Obviously the HCl titer of $0.1N$ HCl is 3.647 mg. A number of other titer values are given in the official description of $0.1N$ HCl; *e.g.*, 8.307 mg. of calcium citrate (anhydrous), 3.705 mg. of calcium hydroxide, etc. Such titer values are of use in the interpretation of analytical data obtained using $0.1N$ HCl.

A twentieth-molar, $0.05M$, solution of sulfuric acid contains 0.05

mole or 4.904 Gm. of H_2SO_4 per liter. Since 1 mole of H_2SO_4 is 2 equivalents, $0.05M$ H_2SO_4 contains $2 \times 0.05 = 0.1$ equivalent per liter and is, therefore, $0.1N$. The normality of a solution can be calculated from its molarity by multiplying the latter by the number of equivalents per mole of the solute.

The importance of a correct evaluation of equivalents in terms of mole fractions or grams in all calculations involving standard solutions may be seen in the following examples. (1) The preparation of 1 liter of $1N$ H_2SO_4 requires 49.04 Gm. of H_2SO_4 since 1 equivalent of H_2SO_4 is needed and since 1 mole or 98.08 Gm. of H_2SO_4 is two equivalents. (2) Ten per cent H_2SO_4 is $1.02M$ or $2.04N$ since 1 liter contains 100 Gm., $100/49.04 = 2.04$ equivalents, or $2.04/2 = 1.02$ moles. (3) Each cubic centimeter of $0.1N$ H_2SO_4 is equivalent to 1.703 mg. of NH_3 since each cubic centimeter of the solution contains 0.0001 equivalent of H_2SO_4, which is equivalent to $0.0001 \times 17.03 = 0.001703$ Gm. or 1.703 mg. of NH_3. In the last example, typical of the calculation of the titer of a solution, 17.03 Gm. was taken as the equivalent weight of NH_3 for the reason indicated by the following equation:

$$\underset{17.03}{NH_3} + H^+ \longrightarrow NH_4^+$$

In practical analytical work, it is convenient to use a solution which has a normality that may be approximately that called for in the analytical procedure. The exact normality of the solution employed, usually expressed to the fourth decimal place, should, however, be known. For example, if $0.1N$ solution is called for, a $0.0965N$ solution, which may be available, is used. From the volume of $0.0965N$ solution employed, the corresponding volume of $0.1N$ solution is readily calculated. Thus, if 20.42 cc. of $0.0965N$ solution were used, the corresponding volume of $0.1N$ solution would be slightly less since the $0.1N$ solution is more concentrated, *viz.*,

$$20.42 \times \frac{0.0965}{0.1} = 19.71 \text{ cc.}$$

A simple rule which facilitates calculations of this type may be deduced from the above calculation:

$$\text{cc.}_a \times N_a = \text{cc.}_b \times N_b$$

It is further apparent that the product of the volume of a standard solution and its normality is the corresponding volume of a 1*N* solution.

In working problems or interpreting data, it is generally expedient to calculate the titer of a 1*N* solution, even though in the assay procedures given in the official compendiums the titer of the solution used, be it 1*N*, 0.1*N*, 0.02*N*, or otherwise, is given. For example, 35.00 cc. of 0.0952*N* HCl was required to neutralize the $Ca(OH)_2$ in a sample of calcium hydroxide solution. Calculate the amount of $Ca(OH)_2$ in the sample.

1. The official procedure specifies that each cubic centimeter of 0.1*N* HCl is equivalent to 3.705 mg. of $Ca(OH)_2$. If this titer value is used, the following calculations are necessary:

$$\frac{35.00 \times 0.0952}{0.1} = 33.32 \text{ cc. of } 0.1N \text{ HCl}$$

$$33.32 \times 3.705 = 123.55 \text{ mg. of } Ca(OH)_2$$

2. If the titer value for 1*N* HCl, 37.05 mg. of $Ca(OH)_2$, is used, the following calculations are necessary:

$$35.00 \times 0.0952 = 3.332 \text{ cc. of } 1N \text{ HCl}$$

$$3.332 \times 37.05 = 123.55 \text{ mg. of } Ca(OH)_2$$

In the latter calculation, (2), the normality factor in the denominator has been eliminated.

The standard acid solutions used in acidimetry and alkalimetry are usually prepared from hydrochloric or sulfuric acid. Either acid may be used in most titrations, but hydrochloric is preferable to sulfuric acid in the titration of compounds that yield a precipitate with the latter, such as barium hydroxide, and sulfuric acid is preferable in hot titrations, since there would be danger of loss of hydrochloric acid due to volatilization.

The standard alkali solutions commonly used are sodium hydroxide, potassium hydroxide, and barium hydroxide. These solutions absorb carbon dioxide from the air, thereby changing rapidly in concentration. Sodium and potassium hydroxides may become contaminated with carbonates that impair their usefulness because of the liberation of carbon dioxide during acid-alkali titration. Barium hydroxide solutions remain free from carbonates, since absorbed carbon dioxide is precipitated as insoluble barium carbonate; this, however, decreases the concentration of barium hydroxide in the solution. Alkali solutions should be prepared carbonate-free, and they should be protected from carbon dioxide by means of soda-lime absorption tubes. All alkali solutions should be restandardized frequently.

Exercise 9

Preparation and Standardization of Hydrochloric Acid Solution. Normal hydrochloric acid solution may be standardized against pure sodium carbonate, standard solution of sodium hydroxide, or gravimetrically by precipitation of an equivalent amount of silver chloride.

Object. To Prepare and Standardize Normal Hydrochloric Acid.

Materials Required. Hydrochloric acid.
4 Gm. of anhydrous sodium carbonate.

Procedure. 1. Dilute 95 cc. of hydrochloric acid withs ufficient distilled water to make 1,000 cc. and mix thoroughly.

Since hydrochloric acid, sp. gr. 1.18, contains about 36 per cent W/W of HCl, the solution prepared according to these directions should be slightly stronger than $1N$. One liter of $1N$ hydrochloric acid should contain 36.47 Gm. or 1 equivalent of HCl. The above solution would contain $95 \times 1.18 \times 0.36 = 40.36$ Gm. of HCl. After standardization, a solution which is slightly greater than $1N$ can be diluted readily to the desired concentration.

2. Weigh about 4 Gm. of reagent anhydrous sodium carbonate on a rough balance and place the salt in a porcelain dish of suitable capacity. Heat the carbonate for 1 hr. over a low Bunsen flame or in an oven at 270° and allow it to cool in a desiccator. Weigh accurately two portions of about 1.5 Gm. each of the cool dry salt, recording the weights.

Sodium carbonate usually contains varying amounts of moisture and sodium bicarbonate. When a sample is heated at 270°, the moisture is driven off and the $NaHCO_3$ is converted to Na_2CO_3.

3. Transfer each of the samples to 250-cc. Erlenmeyer flasks and proceed to titrate them separately as follows: Add about 100 cc. of distilled water and

2 drops of methyl orange T.S. and shake until the carbonate is completely dissolved. Fill the glass-stoppered burette exactly to the mark with the acid solution and run acid slowly into the flask with continuous shaking until the solution just becomes pink in color. A white porcelain plate or a piece of white paper placed beneath the flask renders the end point more easily discernible. Wash down the inside walls of the flask with distilled water from a wash bottle, and if the solution becomes yellow, add acid drop by drop until the faint pink color is restored, taking care that the last drop which adhered to the burette tip is introduced into the solution. When titration is complete, read the burette carefully and record the volume of acid used in the titration.

Assuming that a sample of pure sodium carbonate weighing 1.6250 Gm. required 30.20 cc. of the acid in the titration, the normality of the acid might be calculated as follows: The equivalent weight of sodium carbonate is 106/2 = 53 Gm., because ½ mole of Na_2CO_3 is chemically equivalent to 1 Gm. of hydrogen ions as seen in the following equation:

$$\underset{106}{Na_2CO_3} + 2HCl \longrightarrow 2NaCl + H_2O + CO_2$$

Therefore, 1.6250 Gm. of Na_2CO_3 is 1.6250/53 equivalent of Na_2CO_3. Since 30.20 cc. of the hydrochloric acid solution was found by titration to be chemically equivalent to 1.6250 Gm. of Na_2CO_3, there is 1.6250/53 equivalent of HCl in the 30.20 cc. of hydrochloric acid solution. The number of equivalents of HCl per liter, *i.e.*, normality, may be calculated as follows:

$$\frac{1.6250 \times 1{,}000}{53 \times 30.20} = 1.0152 \text{ equivalent of HCl per liter or } 1.0152N$$

If an exactly normal solution is wanted, the dilution required may be calculated as follows: Assuming that 925.00 cc. of the 1.0152N solution remains after standardization, the corresponding volume of 1N solution would be 925 × 1.0152 = 939.06 cc. and 939.06 − 925.00 = 14.06 cc. of water must be added to the 925.00 cc. of 1.0152N solution to make a 1N solution. The dilution may also be carried out as follows: 7.49 cc. of water is placed in a dry 500-cc. volumetric flask which is then filled to the mark with the 1.0152N solution. After dilution, the solution should be thoroughly mixed and restandardized.

Solutions of hydrochloric acid may also be standardized gravimetrically by using the procedure for the gravimetric determination of a soluble chloride. The equivalent weight of AgCl is 143.34 Gm. If a 20.00-cc. sample of the hydrochloric acid solution yielded 2.9268 Gm. of AgCl, its normality would be

$$\frac{2.9268}{143.34} = \text{equivalents of AgCl formed}$$

$$= \text{equivalents of HCl in 20.00 cc.}$$

$$\frac{2.9268 \times 1{,}000}{143.34 \times 20.00} = \text{equivalents of HCl per liter or normality}$$

It may sometimes be convenient to standardize a solution of hydrochloric acid using a standard solution of NaOH or KOH as a secondary standard. If 25.20 cc. of 0.9505*N* NaOH is required in titration of 25.00 cc. of a solution of hydrochloric acid, the normality of the acid would be $25.20 \times 0.9505/25.00 = 0.9531$.

In analytical procedures, a standard acid of such concentration usually is chosen so that 35 to 40 cc. will be consumed in the titration because volumes in this range can be measured more accurately with a 50-cc. burette than smaller volumes. In the official compendiums, *N*, 0.5*N*, 0.1*N*, 0.05*N*, 0.02*N* or even more dilute solutions of hydrochloric acid are described. The more dilute solutions are conveniently prepared by diluting *N* hydrochloric acid and restandardizing the diluted solution by one of the described methods.

Standard solutions of hydrochloric acid preserved in tightly stoppered alkali-free glass bottles retain their strength indefinitely.

Exercise 10

Preparation and Standardization of Alkali Solutions. Solutions of sodium hydroxide or potassium hydroxide may be standardized by use of a standard solution of hydrochloric or sulfuric acid as a secondary standard, or potassium biphthalate as a primary standard.

Object. To Prepare and Standardize Normal Sodium Hydroxide Solution.

Materials Required. About 50 Gm. of sodium hydroxide.

A saturated solution of barium hydroxide.

A standardized solution of hydrochloric acid.

Procedure. 1. Weigh about 50 Gm. of sodium hydroxide on a rough balance and dissolve it in about 1,000 cc. of distilled water. Add slowly with stirring a saturated solution of pure barium hydroxide until no further precipitation occurs (2 to 3 cc.). Allow the precipitate to subside for about 10 hr. and decant the liquid through a filter into a hard glass bottle or a bottle the inside of which has previously been coated with paraffin. Close the bottle tightly with a rubber stopper provided with a soda-lime tube.

More than 1 equivalent, 40.01 Gm., of sodium hydroxide is weighed out, since it is hygroscopic, and if only 1 equivalent were used the resulting solution would be below normal strength. The solution is treated with barium hydroxide to precipitate any carbonate formed through the action of carbon dioxide on the sodium hydroxide. The solution is preserved in a tightly stoppered bottle fitted with a soda-lime tube to protect it from the carbon dioxide of the air. Solutions which contain carbonate are not suitable for titration with phenolphthalein as indicator, but when methyl orange is used the results are the same as if all the sodium were present, combined as hydroxide.

2. Accurately measure 30 cc. of normal hydrochloric or normal sulfuric acid, dilute with 50 cc. of carbon dioxide-free distilled water, add 2 drops of phenolphthalein T.S., and titrate with the sodium hydroxide solution to the production of a permanent pink color. If the end point is passed in the titration, add a few cubic centimeters more of acid and again titrate to the end point. Titrate several portions of the alkali and from the mean of the results calculate the normality of the sodium hydroxide solution.

A large volume, about 30 cc., of solution is used for each titration to minimize error from measurements. Every precaution should be taken to ensure the correct measurement of the volume of solutions. The burettes should be cleaned, freed from air bubbles before titration, and read with the greatest possible accuracy.

The solution can be made exactly normal by dilution in the same manner as that explained under the preparation of normal hydrochloric acid.

Assuming that 30.00 cc. of 1.0119N hydrochloric acid required 30.15 cc. of the alkali solution, the normality of the latter would be

$$\frac{30.00 \times 1.0119}{30.15} = 1.0069N$$

Standard solutions of potassium hydroxide can be prepared in the same way using 1 mole, 56.10 Gm., of KOH per liter of 1N solution. 0.5N and 0.1N solutions can be prepared directly or by dilution of more concentrated solutions, but they should always be restandardized when prepared by the latter method.

Standardization of sodium hydroxide or potassium hydroxide solutions can also be accomplished by use of reagent potassium biphthalate as a primary standard. As indicated in the following equation, 1 mole or 204.22 Gm. of the pure dried potassium biphthalate is one equivalent.

$$KHC_8H_4O_4 + NaOH \longrightarrow KNaC_8H_4O_4 + H_2O$$

Since it is desirable that approximately 35 cc. of the alkali solution be consumed in a titration of a potassium biphthalate sample, the weight of the potassium biphthalate sample used for standardization of a solution of sodium hydroxide assumed to be 1N can be calculated as follows: 35 cc. of 1N NaOH contains 35 milliequivalents (meq.) of NaOH and is chemically equivalent to 35 meq. of $KHC_8H_4O_4$, or to 35 × 0.20422 = 7.1477 Gm. of $KHC_8H_4O_4$. In the standardization procedure, about 5 Gm. of $KHC_8H_4O_4$ is used. If 24.15 cc. of an alkali solution were required in the titration of a 4.9651-Gm. sample of dried reagent potassium biphthalate, the normality of the solution would be

$$\frac{4.9651 \times 1{,}000}{204.22 \times 24.15} = 1.0070N$$

Exercise 11

Object. To Prepare and Standardize Normal Sulfuric Acid.

Materials Required. 30 cc. of sulfuric acid.
Normal sodium hydroxide solution.

Procedure. Add 30 cc. of sulfuric acid in small portions to 1,020 cc. of distilled water with constant stirring. Allow the solution to acquire room temperature. Standardize the solution by titration against normal sodium hydroxide solution using phenolphthalein as indicator. Calculate the normality and write it on the label or adjust the solution to exactly $1N$.

Since sulfuric acid is a dibasic acid, 1,000 cc. of a normal solution should contain $98.08/2 = 49.04$ Gm. Sulfuric acid (95 per cent) has a specific gravity of about 1.83 at 25°. Consequently, 30 cc. of the concentrated acid should contain about $30 \times 1.83 \times 0.95 = 52.15$ Gm. of H_2SO_4. The method of calculating the normality of the solution is similar to that described under the preparation of normal hydrochloric acid (page 99).

The standardization of sulfuric acid solution can also be effected by titration against pure sodium carbonate, as described under the standardization of hydrochloric acid. It may also be standardized gravimetrically by precipitation and weighing an equivalent amount of barium sulfate, as described under the gravimetric determination of sulfate in a soluble sulfate.

Standard solutions of sulfuric acid preserved in tightly stoppered, alkali-free bottles do not deteriorate.

Questions and Problems

1. Define the following terms: (*a*) normality; (*b*) molarity; (*c*) milliequivalent; (*d*) equivalent; (*e*) equivalent weight.

2. Calculate the equivalent weight of each of the following substances and from that, the corresponding titer values of normal hydrochloric acid. Check the latter by reference to the U.S.P.: (*a*) $CaCO_3$; (*b*) CaO; (*c*) $Na_2B_4O_7$; (*d*) $Na_2B_4O_7.10H_2O$; (*e*) $C_2H_8N_2$ (ethylenediamine).

3. What weight of each of the following substances would be required to make 500 cc. of $0.5N$ solution? If the specific gravity is given, calculate the volume also. (*a*) Hydrochloric acid, 36 per cent W/W, sp. gr. 1.18; (*b*) $Ba(OH)_2.8H_2O$; (*c*) sulfuric acid, 96 per cent W/W, sp. gr. 1.84; (*d*) KOH; (*e*) $1M$ sodium hydroxide.

4. Calculate the normality of each of the following solutions: (*a*) 10 per cent W/V sulfuric acid; (*b*) the official diluted ammonia solution; (*c*) hydrochloric acid solution with a Na_2CO_3 titer of 26.5 mg.; (*d*) sodium hydroxide solution, 36 cc. of which is equivalent to 0.7286 Gm. of pure potassium biphthalate; (*e*) hydrochloric acid solution, 30 cc. of which yields 0.4468 Gm. of AgCl.

5. Calculate the molarity of each of the solutions described in problem 4.

6. Calculate the percentage W/V concentrations of each of the solutions described in problem 4.

7. What volume of $0.1N$ solution can be prepared from each of the following? (*a*) 725 cc. of $0.1321N$ hydrochloric acid; (*b*) 30 cc. of hydrochloric acid, 36 per cent *W/W*, sp. gr. 1.18; (*c*) 250 cc. of $0.1235M$ sulfuric acid.

8. What volume of a solution of hydrochloric acid believed to be $0.1N$ would be used in a standardization experiment to produce 0.5 Gm. of AgCl?

9. What weight of primary standard sodium carbonate would be used as sample for standardizing $0.01N$ sulfuric acid, so that approximately 35 cc. of the acid would be used in the titration?

10. What weight of 98.5 per cent $KHC_8H_4O_4$ would be equivalent to (*a*) 35 cc. of $0.01N$ NaOH; (*b*) 35 cc. of $0.1M$ $Ba(OH)_2$?

11. A 50.00-cc. sample of calcium hydroxide solution required 19.50 cc. of $0.1050N$ hydrochloric acid in a titration. Calculate the amount of $Ca(OH)_2$ in the 50.00-cc. sample and the $Ca(OH)_2$ concentration in per cent *W/V*.

12. Calculate (*a*) the weight; (*b*) the number of moles; (*c*) the volume of $0.1N$ sulfuric acid required to neutralize 0.5 Gm. of CaO.

13. What volume of (*a*) *N*; (*b*) $0.8765N$; (*c*) $0.2M$; (*d*) 10 per cent *W/V* sulfuric acid would be required to neutralize 100 cc. of $1.0560N$ sodium hydroxide?

14. What is the weight in grams of 6.06×10^{23} molecules of HCl? Of 6.06×10^{22} molecules? Of 6.06×10^{10} molecules? Of 7.5×10^{23} molecules?

15. How many molecules of HCl are present in (a) 36.45 Gm., (*b*) 0.3645 Gm., (*c*) 1 Gm., and (*d*) ¼ Gm.?

16. What is the weight in grams of 6.06×10^{23} molecules of NaOH? How many gram molecules are in 40 Gm. of NaOH? How many molecules and how many gram molecules are in 4 Gm. of NaOH?

17. How many molecules of HCl will react with 75 molecules of NaOH? With 6.06×10^{23} molecules?

18. What is the calculated pH of a mixture of 500 cc. of 5 per cent *W/V* sodium hydroxide and 500 cc. of 5 per cent *W/V* hydrochloric acid? What volume of $0.1N$ hydrochloric acid or $0.1N$ sodium hydroxide would be required to adjust the pH to 7?

19. If successive 5-cc. portions of $0.1N$ sodium hydroxide are added to 25.00 cc. of $0.1N$ hydrochloric acid, what would be the pH after each addition?

20. If 25.00 cc. of a hydrochloric acid solution were required in titration of a 0.6500-Gm. sample of pure sodium carbonate, what weight of each of the following pure reagents would be required to neutralize 25 cc. of the hydrochloric acid solution? (*a*) $NaHCO_3$; (*b*) KOH; (*c*) $Ba(OH)_2$; (*d*) CaO.

21. If 25.00 cc. of a sodium hydroxide solution required 40.00 cc. of a sulfuric acid solution in a titration, and 25.00 cc. of the sulfuric acid solution were required in titration of 0.3060 Gm. of pure sodium carbonate, what was the normality of the sodium hydroxide solution?

22. If 50.00 cc. of a hydrochloric acid solution yielded 0.7167 Gm. of AgCl and 30.00 cc. of the same solution were required in titration of 25.00 cc. of a sodium hydroxide solution, what was the normality of the sodium hydroxide solution?

23. A solution of sulfuric acid was standardized gravimetrically and 0.3059 Gm. of barium sulfate was produced from 25.00 cc. of the solution. How much water must be added to 1 liter of the solution to produce a solution exactly 0.1*N*?

24. What weight of barium sulfate would be produced from 10.00 cc. of a sulfuric acid solution if 50.00 cc. of the same solution were found to be equivalent to 0.2650 Gm. of pure sodium carbonate?

25. If 25.50 cc. of a sulfuric acid solution were found to be equivalent to 20.50 cc. of a sodium hydroxide solution, and 35.00 cc. of the sodium hydroxide solution were found to be equivalent to 0.7140 Gm. of pure potassium biphthalate, what weight of barium sulfate could be produced from 50.00 cc. of the sulfuric acid solution?

Chapter 5

ALKALIMETRY

Alkalimetry, the direct or residual titrimetric analysis of bases, is applied in the analytical control of a considerable number of official substances, both inorganic and organic.

Direct titration is conducted by introducing a standard acid solution gradually from a burette into a solution of the base being assayed until chemically equivalent amounts of each have reacted as shown by some change in properties of the mixture. Such an end point, which must be close to the stoichiometric point, is made evident by a change in the color of some indicator or by potentiometric means.

Residual titration is used whenever the end point of a direct titration deviates appreciably from the stoichiometric point for some reason; *e.g.*, when the basic sample is insoluble in water and the rate of its reaction with the standard acid is relatively slow. In such a procedure, the basic sample is treated with an amount of standard acid known to be in excess of a chemically equivalent amount, and the excess acid is subsequently titrated with a standard base. The amount of the standard acid which is chemically equivalent to the basic sample can be calculated simply by subtracting, from the total amount of standard acid employed, the amount of acid that was neutralized by the standard base in the "back titration."

Exercise 12

Object. Assay of Sodium Bicarbonate.

Materials Required. About 3 Gm. of sodium bicarbonate.
Normal sulfuric acid.
Methyl orange T.S.

Procedure. "Weigh accurately about 3 Gm. of Sodium Bicarbonate, previously dried over sulfuric acid for 4 hours, mix it with 25 cc. of water, and titrate with 1*N* sulfuric acid, using methyl orange T.S. as the indicator. Each cubic centimeter of 1*N* sulfuric acid is equivalent to 84.02 mg. of $NaHCO_3$."

It is a general rule that, when the substance to be assayed is a solid, the accurately weighed quantity to be used should be dissolved in sufficient water to make the solution of about the same equivalent concentration as that of the acid to be used in the titration. Methyl orange is used as indicator because phenolphthalein and most other indicators are affected by the carbonic acid liberated in the reaction, which causes a change of color before the reaction is complete:

$$\underset{2(84.02)}{2NaHCO_3} + H_2SO_4 \longrightarrow Na_2SO_4 + 2H_2O + 2CO_2$$

From the equation it is obvious that 2 moles of $NaHCO_3$ are equivalent to 1 mole of H_2SO_4. Therefore 1 mole of $NaHCO_3$ is 1 equivalent and the equivalent weight is equal to the gram-molecular weight, 84.02 Gm. One cc. of 1*N* sulfuric acid or 1 meq. is equivalent to 84.02 mg. or 1 meq. of $NaHCO_3$.

Calculate the percentage purity of the sample assayed.

Questions and Problems

1. Write ionically all equations for all reactions that occur in the above assay.

2. Could normal acetic acid be used to titrate the bicarbonate in place of sulfuric acid?

3. Look up the pH at which methyl orange exhibits its acid color and explain why it is a good indicator to use in this titration.

4. If exactly 3.0 Gm. of $NaHCO_3$ are dissolved in 25 cc. of water in the above assay, what will be the normality of the solution? Does the normality conform to the general rule stated in the discussion?

5. If a 0.2800-Gm. sample of sodium bicarbonate (96.5 per cent $NaHCO_3$) is titrated with 0.9165*N* sulfuric acid, what volume of the acid should be required to produce an end point?

6. Calculate the following titer values for 1*N* sulfuric acid: (*a*) $KHCO_3$; (*b*) K_2CO_3; (*c*) Na_2CO_3. Check the calculated values with those appearing in the U.S.P. or N.F. assay procedures for these substances.

Exercise 13

Object. Assay of Sodium Hydroxide.

Materials Required. 1.5 Gm. of sodium hydroxide.
Normal sulfuric acid.

Procedure. 1. "Dissolve about 1.5 Gm. of Sodium Hydroxide, accurately weighed, in about 40 cc. of recently boiled and cooled water. Cool the solution to 15°, and titrate with 1*N* sulfuric acid, using phenolphthalein T.S. as the indicator. At the discharge of the pink color of the indicator, record the volume of acid solution required."

The base, NaOH, behaves in the same manner when either phenolphthalein or methyl orange is used as the indicator. Consequently, the end point obtained with phenolphthalein as the indicator represents complete neutralization of all the NaOH as represented by the equation:

$$\begin{array}{l} 2NaOH + H_2SO_4 \longrightarrow Na_2SO_4 + 2H_2O \\ 2(40.01) \end{array} \qquad (1)$$

In a cold solution, with phenolphthalein indicator, the end point of titration of sodium carbonate with standard H_2SO_4 is exhibited when the solution is half neutralized or when the Na_2CO_3 is transformed into $NaHCO_3$.

$$\begin{array}{l} 2Na_2CO_3 + H_2SO_4 \longrightarrow Na_2SO_4 + 2NaHCO_3 \\ 2(106) \end{array} \qquad (2)$$

2. "Add 3 drops of methyl orange T.S., and continue the titration to the production of a persistent pink color. Each cubic centimeter of 1*N* sulfuric acid consumed is equivalent to 40.01 mg. of NaOH. Each cubic centimeter difference between the number of cubic centimeters of normal sulfuric acid consumed in the methyl orange and phenolphthalein titrations is equivalent to 106.0 mg. of Na_2CO_3."

In titration to a phenolphthalein end point, NaOH is neutralized and Na_2CO_3 is converted to $NaHCO_3$. As the Na_2CO_3 is converted to $NaHCO_3$ ($CO_3^{=} + H^+ \rightleftharpoons HCO_3^-$), the ($H^+$) remains low because the $CO_3^{=}$ ion is strongly basic. Consequently, the pH of the mixture remains above the range 8 to 9.8 over which phenolphthalein changes color, until the conversion of Na_2CO_3 to $NaHCO_3$ is complete. The HCO_3^- ion is weakly basic ($HCO_3^- + H^+ \rightleftharpoons H_2CO_3$) and the $NaHCO_3$ formed remains unneutralized even though (H^+) has been increased to the point where phenolphthalein undergoes a change in color. The neutralization of the $NaHCO_3$ is complete only when (H^+) has been increased to the point where methyl orange undergoes a change in color at pH 3.1 to 4.4.

The reaction is indicated by the equation:

$$\underset{2(84.02)}{2NaHCO_3} + H_2SO_4 \longrightarrow Na_2SO_4 + 2CO_2 + 2H_2O \qquad (3)$$

The total volume of normal sulfuric acid used in the titration was required to neutralize NaOH and Na_2CO_3, converting the latter first to $NaHCO_3$ at the phenolphthalein end point and then to H_2CO_3 at the methyl orange end point. Although part of the standard acid was actually neutralized by Na_2CO_3, the N.F. gives specifications for *total alkali* including NaOH and Na_2CO_3, calculated as NaOH. From Eq. (1), it can be seen that the equivalent weight of NaOH is 40.01 Gm. Therefore, each cubic centimeter of the total amount of $1N$ acid consumed is equivalent to 40.01 mg. or 1 meq. of NaOH.

The difference between the total volume of standard acid used and the volume necessary to produce a phenolphthalein end point gives the volume of standard acid necessary to neutralize the $NaHCO_3$ formed from the Na_2CO_3 during titration to the phenolphthalein end point. This same volume was also necessary to convert the Na_2CO_3 to $NaHCO_3$ according to Eq. (2). From this equation, the equivalent weight of Na_2CO_3 is calculated as 106.0 Gm. Consequently, each cubic centimeter of the acid volume calculated by difference is equivalent to 106.0 mg. or 1 meq. of Na_2CO_3.

Interpretation of the following data will illustrate the calculations involved. A 965.4-mg. sample of sodium hydroxide required 20.80 cc. of $1N$ sulfuric acid in titration to a phenolphthalein end point, and 21.75 cc. of $1N$ sulfuric acid in titration to a methyl orange end point. The 21.75 cc. of $1N$ sulfuric acid consumed is a measure of the total alkalinity, NaOH plus Na_2CO_3. In order to express total alkalinity in terms of NaOH, it is assumed that each cubic centimeter of the $1N$ acid is equivalent to 40.01 mg. of NaOH. The total alkalinity calculated as NaOH is therefore

$$\frac{21.75 \times 40.01 \times 100}{965.4} = 90.12 \text{ per cent}$$

The volume of $1N$ sulfuric acid, $21.75 - 20.80 = 0.95$ cc., required to neutralize the $NaHCO_3$ as in Eq. (3) is equal to the volume necessary to produce the $NaHCO_3$ from Na_2CO_3 as in Eq. (2). From Eq. (2), it can be calculated that each cubic centimeter of $1N$ sulfuric acid is equivalent to 106.0 mg. of Na_2CO_3. Therefore the proportion of Na_2CO_3 in the sample is

$$\frac{0.95 \times 106.0 \times 100}{965.4} = 10.4 \text{ per cent}$$

Since 0.95 cc. of $1N$ sulfuric acid was required to convert the Na_2CO_3 to $NaHCO_3$ and an equal volume to neutralize the $NaHCO_3$, then $2 \times 0.95 = 1.90$ cc. of $1N$ sulfuric acid was required for complete neutralization of the Na_2CO_3 according to the following equation, which is a summation of Eq. (2) and (3):

$$\underset{106.0}{Na_2CO_3} + H_2SO_4 \longrightarrow Na_2SO_4 + H_2O + CO_2$$

The equivalent weight of Na_2CO_3, calculated from this equation, is 53.0 Gm. Each cubic centimeter of $1N$ sulfuric acid required for complete neutralization of Na_2CO_3 is equivalent to 53.0 mg. of Na_2GO_3. Since 1.90 cc. was required, the proportion of Na_2CO_3 in the sample is

$$\frac{1.90 \times 53.0 \times 100}{965.4} = 10.4 \text{ per cent}$$

Questions and Problems

1. In titration of a 1.0900-Gm. sample of sodium carbonate (98.5 per cent Na_2CO_3) with $0.9050N$ sulfuric acid, what volumes would be necessary to produce (*a*) phenolphthalein end point; (*b*) methyl orange end point?

2. In titration of a mixture of 0.5000 Gm. of pure Na_2CO_3 and 0.4000 Gm. of pure $NaHCO_3$ with $1.1050N$ sulfuric acid, what volume of the acid would be necessary to produce (*a*) phenolphthalein end point; (*b*) methyl orange end point?

3. Calculate the proportion of (*a*) KOH; (*b*) K_2CO_3; (*c*) the total alkalinity in terms of KOH, in a solution of potassium hydroxide, 25.00 cc. of which required 26.00 cc. of $0.8650N$ sulfuric acid in titration to a phenolphthalein end point and 31.15 cc. of the same acid in titration to a methyl orange end point.

4. Calculate the Na_2CO_3 and $NaHCO_3$ content of a 1.2500-Gm. sample requiring 13.80 cc. of $0.5250N$ hydrochloric acid on titration to a phenolphthalein end point and an additional 16.30 cc. on further titration to a methyl orange end point.

Exercise 14

Object. Assay of Sodium Salicylate.

Materials Required. 2 Gm. of sodium salicylate.
100 cc. of ether.
0.5*N* hydrochloric acid.
Bromophenol blue T.S.

Procedure. 1. "Weigh accurately about 2 Gm. of Sodium Salicylate, previously dried at 105° for 4 hours, and dissolve it in about 25 cc. of water in a separator. Add 75 cc. of ether and 10 drops of bromophenol blue T.S., and titrate with 0.5*N* hydrochloric acid, mixing the water and ether layers intimately by vigorous shaking until a permanent pale green color is produced in the water layer."

Sodium salicylate, like sodium carbonate and sodium bicarbonate, is a salt of a strong base and a weak acid, and will neutralize hydrochloric acid by a reaction ($C_7H_5O_3^- + H^+ \rightleftharpoons HC_7H_5O_3$) which is practically complete when (H^+) has been increased to the point where bromophenol blue undergoes a change in color at pH 3.0 to 4.6. The salicylic acid which is formed is not soluble in water, but the ether dissolves it and thus makes possible observation of the color change in the aqueous phase that would otherwise be obscured by the precipitated salicylic acid. For purposes of stoichiometry, the following equation is used:

$$\underset{160.11}{C_7H_5NaO_3} + HCl \longrightarrow HC_7H_5O_3 + NaCl$$

2. "Draw off the water layer into a small flask, wash the ether layer once with 5 cc. of water, and add this to the water layer. Add 20 cc. of ether to the combined water solutions, and mix intimately. Continue the titration with vigorous shaking until a permanent, pale green color is produced in the water layer. Each cubic centimeter of 0.5*N* hydrochloric acid is equivalent to 80.05 mg. of $C_7H_5NaO_3$."

The separated ethereal layer is washed with water to remove small amounts of sodium salicylate that may remain dissolved therein. Upon washing the combined aqueous solutions and mixing intimately, any remaining salicylic acid partitions itself into the ethereal layer, leaving only the HCl in the aqueous layer to influence the end point.

Questions and Problems

1. What are the advantages of bromophenol blue as indicator in direct titrimetric analysis of sodium salicylate?

2. In what ways is the assay procedure described for sodium salicylate modified when applied in assay of sodium salicylate tablets?

3. Consult Table 14 and make a list of official preparations in which the salt of an organic acid is assayed by direct alkalimetry. Calculate the 0.5*N* hydrochloric acid titer values for each of the following and compare with values given in the U.S.P. or N.F.: (*a*) sodium benzoate; (*b*) sodium glycerophosphate; (*c*) strontium salicylate.

4. The disodium salt of a dibasic organic acid has a molecular weight of 215; what weight of sample would be used for titration to a bromophenol blue end point with 0.2500*N* acid in order that 35.00 cc. of the latter will be required?

5. Twenty sodium salicylate tablets, labeled 5-grain, were dispersed in sufficient water to make 200.00 cc. A 15.00-cc. aliquot part of the filtered aqueous dispersion was titrated to a bromophenol blue end point in the usual way by 29.11 cc. of 0.1000*N* hydrochloric acid. Calculate the amount of sodium salicylate in each tablet and, from that, the percentage of the labeled amount. Note: 1 grain = 65 mg.

RESIDUAL TITRATION METHODS

Residual titration or "back titration" is frequently used when a reaction proceeds slowly or when the substance to be assayed does not give a distinct, sharp end point with an indicator by direct titration. Residual titration is carried out by dissolving the substance under examination in an accurately measured quantity of standard solution known to be in excess and titrating the excess of the latter with another standard solution. Numerous examples of this method of titration will be met with in subsequent exercises.

Exercise 15

Object. Assay of Zinc Oxide.

Materials Required. 1.5 Gm. of zinc oxide.
Normal sulfuric acid.
Normal sodium hydroxide.

Procedure. "Dissolve about 1.5 Gm. of freshly ignited Zinc Oxide, accurately weighed, and 2.5 Gm. of ammonium chloride in 50 cc. of 1*N* sulfuric acid with the aid of gentle heat, if necessary. When solution is complete, titrate

Table 14. Official Alkalimetric Assays by Direct Titration

Substance	Normality of acid	Indicator	Equivalent of 1 cc., mg.	Official requirement, per cent
U.S.P.				
Aminophylline	0.1	M.O.	3.005	$C_2H_8N_2 = 12.3$ to 13.8
Aminophylline injection	0.1	M.O.	3.005	$C_2H_8N_2 = 60$ mg. per Gm. of Aminophylline
Ammonia solution, diluted	1.0	M.R.	17.03	$NH_3 = 9$ to 10*W/V*
Ammonia solution, strong	1.0	M.R.	17.03	$NH_3 = 27$ to 30
Ammonia spirit, aromatic	0.5	Pp., M.O.	48.05	$(NH_4)_2CO_3 \doteq 3.5$ to 4.5*W/V*
Caffeine and sodium benzoate	0.1	M.O.	14.41	$C_7H_5NaO_2 = 50$ to 53
Caffeine and sodium benzoate injection	0.1	M.O.	14.41	$C_7H_5NaO_2 = 47.5$ to 55.5*
Calcium hydroxide solution	0.1	Pp.	3.705	$Ca(OH)_2 =$ (n.l.t.) 0.14*W/V*
Ethylenediamine solution	1.0	B.P.B.	30.05	$C_2H_8N_2 =$ (n.l.t.) 67
Potassium bicarbonate	1.0	M.O.	100.1	$KHCO_3 =$ (n.l.t.) 99
Potassium hydroxide	1.0	Pp., M.O.	56.10	$KOH =$ (n.l.t.) 85
			138.2	$K_2CO_3 =$ (n.m.t.) 3.5
Sodium benzoate	0.5	M.O.	72.06	$C_7H_5NaO_2 =$ (n.l.t.) 99
Sodium bicarbonate	1.0	M.O.	84.02	$NaHCO_3 =$ (n.l.t.) 99
Sodium bicarbonate tablets	1.0	M.O.	84.02	$NaHCO_3 = 95$ to 105*
Sodium borate	0.5	M.R.	95.36	$Na_2B_4O_7.10H_2O = 99$ to 105
Sodium salicylate	0.5	B.P.B.	80.05	$C_7H_5NaO_3 =$ (n.l.t.) 99.5
Sodium salicylate injection	0.1	B.P.B.	16.01	$C_7H_5NaO_3 = 95$ to 105*
Sodium salicylate tablets	0.1	B.P.B.	16.01	$C_7H_5NaO_3 = 95$ to 105*
Triethanolamine	1.0	M.R.	....	Alkalinity = 6.7 to 7.2 cc. of 1*N* acid per Gm.
N.F.				
Ammonium salicylate	0.1	B.P.B.	15.52	$C_7H_5NH_4O_3 =$ (n.l.t.) 98
Potassium carbonate	1.0	M.O.	69.10	$K_2CO_3 =$ (n.l.t.) 99
Potassium citrate solution	0.1	M.O.	10.21	$C_6H_5K_3O_7 =$ (n.l.t.) 8*W/V*
Sodium acetate	0.1	M.O.	8.204	$C_2H_3NaO_2 =$ (n.l.t.) 99
Sodium carbonate monohydrated	1.0	M.O.	53.00	$Na_2CO_3 =$ (n.l.t.) 99.5
Sodium citrate solution	0.1	M.O.	9.804	$C_6H_5Na_3O_7.2H_2O = 2.5$–3.0*W/V*
Sodium glycerophosphate	0.5	M.O.	108.0	$C_3H_7Na_2O_6P = 68$ to 74
Sodium hydroxide	1.0	Pp., M.O.	40.01	$NaOH =$ (n.l.t.) 95
			106.0	$Na_2CO_3 =$ (n.m.t.) 3
Sodium propionate	0.1	M.O.	9.607	$C_3H_5NaO_2 =$ (n.l.t.) 99
Sodium salicylate and iodide injection	0.1	B.P.B.	16.01	$C_7H_5NaO_3 = 93$ to 103*
Sodium salicylate injection	0.1	B.P.B.	16.01	$C_7H_5NaO_3 = 8$ to 9*W/V*
Strontium salicylate	0.5	M.O.	99.47	$C_{14}H_{10}O_6Sr.2H_2O =$ (n.l.t.) 99

* Per cent of labeled amount.

M.O. = methyl orange, M.R. = methyl red, Pp. = phenolphthalein, B.P.B. = bromophenol blue.

(n.l.t.) = not less than; (n.m.t.) = not more than.

the excess sulfuric acid with 1*N* sodium hydroxide, using methyl orange T.S. as the indicator. Each cubic centimeter of 1*N* sulfuric acid is equivalent to 40.69 mg. of ZnO."

The ZnO dissolves in the sulfuric acid, neutralizing an equivalent amount in accordance with the equation

$$\underset{81.38}{ZnO} + H_2SO_4 \longrightarrow ZnSO_4 + H_2O$$

The amount of sulfuric acid neutralized by the ZnO in this way is determined simply by subtracting, from the total amount of sulfuric acid used, the amount that was neutralized by the standard NaOH in the back titration. The equivalent weight of ZnO, as indicated by the equation, is 40.69 Gm.; therefore, each cubic centimeter of 1*N* sulfuric acid, 1 meq., neutralized by the ZnO is equivalent to 40.69 mg. or 1 meq. of ZnO.

Calculate the percentage of zinc and the percentage of zinc oxide in the sample assayed.

Questions and Problems

1. Suggest a reason for the use of ammonium chloride in the assay of zinc oxide. In titration of the mixture to a methyl orange end point with standard sodium hydroxide, why is none of the NaOH neutralized by the NH_4Cl?

2. Give a reason explaining why the standard acid used in assay of zinc oxide is normal while that used in assay of zinc stearate is only 0.1*N*. Calculate the weight of zinc stearate that would be used as sample if normal sulfuric acid were employed, so that 35.00 cc. of the acid would be neutralized by the zinc stearate.

3. If a 1.2500-Gm. sample of zinc oxide, 95.0 per cent of ZnO, were treated with 50.00 cc. of 1.1230*N* sulfuric acid in the usual way, what volume of 0.9765*N* sodium hydroxide would be required in the back titration?

Exercise 16

Determination of the Purity of the Alkali Salt of an Organic Acid. In Table 14 are included a number of alkali salts of organic acids which are assayed by a direct alkalimetric procedure. A number of others, however, are first ignited to an equivalent amount of carbonate and this carbonate in the ash formed by ignition is determined residually. Certain official acetates, lactates, tartrates, and citrates are assayed in this way and the official compendiums give general directions for the assay of "Alkali Salts of Organic Acids."

Object. Assay of Potassium Sodium Tartrate.

Materials Required. 3 Gm. of potassium sodium tartrate.
0.5*N* sulfuric acid.
0.5*N* sodium hydroxide.

Procedure. 1. "Dry about 3 Gm. of Potassium Sodium Tartrate, accurately weighed, at 150° for 3 hours; it loses not less than 21 per cent and not more than 26 per cent of its weight."

The $C_4H_4KNaO_6$ content of potassium sodium tartrate is controlled first by specifications pertaining to the water content and then by specifications of the $C_4H_4KNaO_6$ content of the dried material. The latter determination is made according to the general procedure for assay of alkali salts of organic acids, using the material dried as described above.

2. "Heat about 2 Gm. of the organic salt, accurately weighed, in a platinum or porcelain crucible (do not use platinum for lithium salts), at first very gently, then gradually raise the temperature until the salt is thoroughly carbonized. The final temperature must not exceed a dull red heat. If a burner is used, the flame must not come in contact with the carbonized mass."

The salt is heated slowly at first, since the sample swells and fuses with concomitant decomposition. If strong heat is applied in the initial stages of ignition, there may be loss of a portion of the sample through decrepitation or spattering. After the mass in the crucible is partially charred and white fumes are no longer evolved, it is ignited to dull redness. If heated too strongly or if the flame comes in contact with the carbonized mass, the alkali carbonate may be converted into the oxide.

3. "After allowing the carbonized mass to cool, disintegrate it with the aid of a stout glass rod, and transfer the mass and the crucible to a beaker. Add 50 cc. of water and exactly 50 cc. of 0.5*N* sulfuric acid, cover the beaker with a watch glass, and boil the contents for 30 minutes. Filter the solution, and wash the residue with hot water until the washings cease to redden blue litmus paper. Determine the residual acid in the cooled filtrate by titration with 0.5*N* sodium hydroxide, using methyl orange T.S. as the indicator. The volume of the acid consumed, multiplied by the proper equivalent of the salt, represents the quantity of the salt present in the quantity taken."

The fused residue is broken up by means of a strong glass rod over glazed, black paper and transferred with the crucible into a 250-cc. beaker. About 50 cc. of distilled water and 50 cc. of sulfuric acid are added along the glass rod, and the mass is completely dissolved.

During the ignition of the tartrate, an equivalent amount of carbonate is formed,

$$\underset{210.17}{C_4H_4KNaO_6} \xrightarrow{\text{air}} KNaCO_3$$

and this neutralizes an equivalent amount of the acid with which the ash is treated.

$$KNaCO_3 + H_2SO_4 \longrightarrow KNaSO_4 + H_2O + CO_2$$

It is obvious from the equations that the carbonate from 1 mole of $C_4H_4KNaO_6$ will neutralize 2 equivalents, 1 mole, of sulfuric acid; therefore, the equivalent weight of $C_4H_4KNaO_6$ is 105.09 Gm. Each cubic centimeter of $0.5N$ sulfuric acid, 0.5 meq., is equivalent to $0.5 \times 0.10509 = 0.05245$ Gm. or 0.5 meq. of $C_4H_4KNaO_6$.

The N.F. requires that potassium sodium tartrate, when rendered anhydrous by drying at 150°, contain not less than 99 per cent of $C_4H_4KNaO_6$. Calculate the purity of the material obtained on drying in terms of $C_4H_4KNaO_6$.

Questions and Problems

1. Why cannot the alkali salts of such acids as citric, tartaric, etc., be titrated directly with a strong acid in the same manner as soluble carbonates are titrated?

2. Calculate the weight of each of the following substances which produces sufficient carbonate on ignition to neutralize 1 cc. of $0.5N$ sulfuric acid: *(a)* sodium citrate, $C_6H_5Na_3O_7$; *(b)* potassium acetate, $C_2H_3KO_2$; *(c)* potassium citrate, $C_6H_5K_3O_7$. Compare with the titer values of $0.5N$ sulfuric acid given in U.S.P. and N.F.

3. What weight of potassium acetate containing 91.5 per cent $C_2H_3KO_2$ must be used as sample in an assay according to the general method for alkali salts of organic acids if, after treatment of the ash with 50.00 cc. of $0.5162N$ sulfuric acid, it is desirable to consume 30.00 cc. of $0.4675N$ sodium hydroxide in the back titration?

4. A mixture of sodium bicarbonate and potassium sodium tartrate (compound effervescent powders, blue paper) was assayed for $NaHCO_3$ and $KNaC_4H_4O_6.4H_2O$. A 2.2440-Gm. sample was treated with 20.00 cc. of 0.5N sulfuric acid and the mixture titrated to a phenolphthalein end point with 6.17 cc. of 0.5N sodium hydroxide. A 1.8760-Gm. sample was ignited, the ash taken up in 50.00 cc. of 0.5N sulfuric acid, and the mixture titrated to methyl orange end point with 19.84 cc. of 0.5N sodium hydroxide. Calculate the $NaHCO_3$ and $KNaC_4H_4O_6.4H_2O$ content of the material.

Exercise 17

Object. Assay of Magnesia Magma.

Materials Required. 5 Gm. of magnesia magma.
1N sulfuric acid.
1N sodium hydroxide.

Procedure. "After thorough agitation, place about 5 Gm. of Magnesia Magma in a tared flask, stopper, weigh accurately, add 25 cc. of 1N sulfuric acid, and after solution is complete, titrate the excess acid with 1N sodium hydroxide, using methyl red T.S. as the indicator. Each cubic centimeter of 1N sulfuric acid is equivalent to 29.17 mg. of $Mg(OH)_2$."

The magnesia magma is dissolved in an accurately measured excess of 1N sulfuric acid solution to ensure complete neutralization of all the magnesium hydroxide with the formation of the soluble magnesium sulfate. The excess acid is then determined by residual titration with 1N sodium hydroxide, using methyl red as indicator.

The reactions which take place when the magnesia magma is dissolved and upon residual titration are as follows:

$$\underset{58.34}{Mg(OH)_2} + H_2SO_4 \longrightarrow MgSO_4 + 2H_2O$$

$$H_2SO_4 + 2NaOH \longrightarrow Na_2SO_4 + 2H_2O$$

The equivalent weight of $Mg(OH)_2$ is 29.17 Gm.; consequently each cubic centimeter of 1N sulfuric acid neutralized by $Mg(OH)_2$ is equivalent to 29.17 mg. of $Mg(OH)_2$.

If a sample of magnesia magma weighing 5.2430 Gm. when dissolved in 25 cc. of 0.9915N H_2SO_4 required 9.85 cc. of 1.1402N NaOH to titrate the excess acid, the per cent of $Mg(OH)_2$ in the sample could be calculated from the following formula:

$$\frac{[(\text{cc. of } H_2SO_4 \times N) - (\text{cc. of NaOH} \times N)] \times \text{equivalent}}{\text{Wt. of sample}}$$

× 100 = per cent, or substituting the figures from the example cited above,

$$\frac{[(25 \times 0.9915) - (9.85 \times 1.1102)] \times 0.02917}{5.2430} \times 100$$

$$= 7.54 \text{ per cent}$$

The U.S.P. requires that magnesia magma contain not less than 7 and not more than 8.5 per cent of $Mg(OH)_2$. The above sample, therefore, conforms to the official requirements.

Questions and Problems

1. Compare the assay procedures employed for magnesium sulfate, magnesium hydroxide, and magnesia magma, giving equations for all reactions involved.

2. If 0.5*N* sulfuric acid were used in assay of magnesia magma, how would the U.S.P. directions be modified?

3. Calculate the MgO content of magnesia magma, 12.32 Gm. of which was dissolved in 50.00 cc. of 1.0340*N* sulfuric acid, producing a mixture that required 24.60 cc. of 1.1255*N* sodium hydroxide.

Exercise 18

Object. Assay of Methenamine.

Materials Required. 1 Gm. of methenamine.
1*N* sulfuric acid.
1*N* sodium hydroxide.

Procedure. 1. "Weigh accurately about 1 Gm. of Methenamine, previously dried over sulfuric acid for 4 hours, place it in a beaker, add 40 cc. of 1*N* sulfuric acid, and boil gently, adding water from time to time, if necessary, until the odor of formaldehyde is no longer perceptible."

The weighed sample of methenamine is heated on a water bath with a measured excess of 1*N* sulfuric acid to decompose it into ammonia and formaldehyde, water being added from time to time to replace the water lost by evaporation. Completion of the decomposition is indicated when the odor of formaldehyde is no longer perceptible. The decomposition reaction may be indicated in two steps as follows:

$$\underset{140.19}{(CH_2)_6N_4} + 10H_2O \longrightarrow 6CH_2O + 4NH_4OH$$

$$4NH_4OH + 2H_2SO_4 \longrightarrow 2(NH_4)_2SO_4 + 4H_2O$$

2. "Cool, add 20 cc. of water, and titrate the excess acid with 1*N* sodium hydroxide, using methyl red T.S. as the indicator. Each cubic centimeter of 1*N* sulfuric acid is equivalent to 35.05 mg. of $C_6H_{12}N_4$."

The excess sulfuric acid found by residual titration with 1*N* sodium hydroxide, subtracted from the amount originally added, gives the amount of acid that combined with the liberated ammonia.

A summation of the above two equations indicates the equivalence of 1 mole of methenamine and 2 moles of sulfuric acid; hence ¼ mole, 35.05 Gm., of methenamine is one equivalent and each cubic centimeter of 1*N* sulfuric acid is equivalent to 35.05 mg. or 1 meq. of methenamine.

Calculate the percentage purity of the sample assayed.

Questions and Problems

1. Give a reason for the addition of water from time to time to replace that lost on heating the mixture of methenamine and standard sulfuric acid.

2. Suggest a possible advantage in the use of standard sulfuric acid rather than hydrochloric acid in the assay of methenamine.

3. If a 0.4340-Gm. sample of methenamine were treated in the usual way with 25.00 cc. of 0.6500*N* sulfuric acid, and 9.45 cc. of 0.4500*N* sodium hydroxide were required in the back titration, what was the per cent $C_6H_{12}N_4$ of the sample?

Exercise 19

Object. Assay of Ammonium Acetate Solution.

Materials Required. 25 cc. of ammonium acetate solution.
Sodium hydroxide T.S.
1*N* sulfuric acid.
1*N* sodium hydroxide.

Procedure. "Transfer 25 cc. of Ammonium Acetate Solution to a distilling flask, dilute with 75 cc. of water, add 50 cc. of sodium hydroxide T.S., and distil the liquid until all of the ammonia has been driven over (about 100 cc. of distillate), receiving the distillate below the surface of 50 cc. of 1*N* sulfuric acid contained in a flask. Titrate the excess of acid with 1*N* sodium hydroxide, using methyl red T.S. as the indicator. Each cubic centimeter of 1*N* sulfuric acid is equivalent to 77.08 mg. of CH_3COONH_4."

The NaOH reacts with the ammonium acetate, liberating NH_3 and combining with the acetic and carbonic acids to form nonvolatile compounds. The distilling flask should be connected to the condenser at once after adding the NaOH to prevent loss of the volatile NH_3. The NH_3 formed in the reaction,

$$\underset{77.08}{CH_3COONH_4} + NaOH \longrightarrow CH_3COONa + NH_3 + H_2O$$

distills with water when the flask is heated. The NH_3 is conducted into a receiving flask which contains an excess of N H_2SO_4. The condenser should be fitted tightly with an adapter in such a manner that the distillate will be collected beneath the surface of the standard acid. This is done to prevent loss of NH_3 from the distillate. As soon as the NH_3 in the distillate comes into the receiver flask, it combines with the H_2SO_4 to form $(NH_4)_2SO_4$,

$$2NH_3 + H_2SO_4 \longrightarrow (NH_4)_2SO_4$$

The excess acid is then determined by titration with standard alkali.

A number of official preparations containing free ammonia, ammonium salts, or ammonia complexes such as ammonia liniment and ammoniacal silver nitrate solution are assayed by methods which in principle resemble the assay of ammonium acetate solution. The ammonia is quantitatively liberated and distilled into standard acid. An important modification of this rather general method which appears in the procedure for ammonium chloride capsules obviates the necessity for using a standard sodium hydroxide solution. In this procedure, the liberated ammonia is distilled into boric acid solution (1 in 25) and is fixed by the weakly acidic boric acid as indicated by the equation

$$NH_3 + H_3BO_3 \rightleftharpoons NH_4BO_2 + H_2O$$

Boric acid is such a weak acid that it has no effect on such indicators as bromocresol green or methyl red, and a sharp end point results when the solution is titrated with a quantity of standard sulfuric or hydrochloric acid equivalent to the NH_3 resulting from complete hydrolysis of ammonium metaborate.

In the assay of a number of official substances such as neostigmine preparations, nicotinamide, methenamine, and sodium biphosphate tablets, ammonia or a volatile amine in organic combination, *e.g.*, as amide or carbamate, is liberated by a quantitative hydrolytic degradation of the organic substance on treatment with sodium hydroxide. Sometimes an acid hydrolysis is employed before the ammonia or amine is liberated by addition of sodium hydroxide as in nikethamide injection, quinine and urethane injection, and saccharin sodium tablets. Frequently, in the assay of these preparations, the distilled ammonia or amine is collected in boric acid solution as described above.

In Table 15, preparations which have assay procedures in which distillation of ammonia or an amine is involved are indicated by an asterisk.

Certain organic bases which are not volatile can be liberated from their salts and extracted with chloroform or ether in a manner analogous to the liberation of ammonia of volatile amine from a salt. After concentration of the solution of the organic base in the organic solvent, a measured excess of standard acid is added. The mixture is heated to complete the removal or organic solvent and the excess acid is determined by back titration with a standard base. Such a procedure is applied in the assay of diphenhydramine hydrochloride, meperidine hydrochloride, naphazoline hydrochloride, piperocaine hydrochloride, and preparations containing the foregoing substances and preparations containing procaine hydrochloride (see Table 15).

Questions and Problems

1. Calculate the pH of a 1*M* solution of ammonium chloride and of a 1 in 25 solution of boric acid, and explain the significance of the values with respect to the titration of ammonia dissolved in boric acid with standard hydrochloric acid using a methyl red indicator.

2. Give equations for the reactions that take place in the assay of each of the following, and calculate the equivalent weight of the substance determined: (*a*) nikethamide injection; (*b*) saccharin sodium tablets; (*c*) neostigmine bromide; (*d*) nicotinamide.

3. The NH_3 from a 0.3256-Gm. sample of acid ammonium valerate was distilled into 25.00 cc. of 0.1342*N* sulfuric acid and the mixture titrated to a methyl red end point with 25.05 cc. of 0.0950*N* sodium hydroxide. Calculate the per cent of $C_4H_9COONH_4$ in the sample.

Determination of Nitrogen Content by the Kjeldahl Method. It frequently happens that ammonia is not quantita-

TABLE 15. OFFICIAL ALKALIMETRIC ASSAYS BY RESIDUAL TITRATION

Substance	Normality of acid	Indicator	Equivalent of 1 cc., mg.	Official requirement, per cent
U.S.P.				
Ammonia spirit, aromatic	0.5	M.R.	8.516	NH_3 (total) = 1.7 to 2.1 W/V
Ammonium carbonate	1.0	M.O.	17.03	NH_3 = 30 to 33
Ammonium chloride capsules*	0.1	M.R.	5.350	NH_4Cl = 93 to 107†
Calamine	1.0	M.O.	40.69	ZnO = (n.l.t.) 98†
Diphenhydramine hydrochloride	0.05	M.R.	14.59	$C_{17}H_{21}NO.HCl$ = (n.l.t.) 98
Diphenhydramine hydrochloride capsules	0.05	M.R.	14.59	$C_{17}H_{21}NO.HCl$ = 93 to 107†
Glyceryl trinitrate tablets*	0.02	M.R.	1.514	$C_3H_5N_3O_9$ = 80 to 112†
Magnesia magma	1.0	M.R.	29.17	$Mg(OH)_2$ = 7 to 8.5
Magnesium carbonate	1.0	M.O.	20.16	MgO = 40 to 43.5
Magnesium oxide	1.0	M.O.	20.16	MgO = (n.l.t.) 96
Magnesium oxide, heavy	1.0	M.O.	20.16	MgO = (n.l.t.) 96
Magnesium trisilicate	1.0	M.O.	20.16	MgO = (n.l.t.) 20
Meperidine hydrochloride	0.05	M.R.	14.19	$C_{15}H_{21}NO_2.HCl$ = (n.l.t.) 97
Meperidine hydrochloride injection	0.02	M.R.	5.676	$C_{15}H_{21}NO_2.HCl$ = 95 to 105†
Meperidine hydrochloride tablets	0.02	M.R.	5.676	$C_{15}H_{21}NO_2.HCl$ = 94 to 106†
Methenamine	1.0	M.R.	35.05	$C_6H_{12}N_4$ = (n.l.t.) 99
Methenamine tablets	1.0	M.R.	35.05	$C_6H_{12}N_4$ = 95 to 105†
Naphazoline hydrochloride	0.05	M.R.	12.34	$C_{14}H_{14}N_2.HCl$ = (n.l.t.) 98
Naphazoline hydrochloride solution, mild	0.02	M.R.	4.935	$C_{14}H_{14}N_2.HCl$ = 0.045 to 0.055 W/V
Naphazoline hydrochloride solution, strong	0.02	M.R.	4.935	$C_{14}H_{14}N_2.HCl$ = 0.093 to 0.107 W/V
Neostigmine bromide*	0.02	M.R.	6.064	$C_{12}H_{19}BrN_2O_2$ = (n.l.t.) 98
Neostigmine bromide tablets*	0.02	M.R.	6.064	$C_{12}H_{19}BrN_2O_2$ = 93 to 107†
Neostigmine methylsulfate*	0.02	M.R.	6.688	$C_{13}H_{22}N_2O_6S$ = (n.l.t.) 98
Neostigmine methylsulfate injection*	0.02	M.R.	6.688	$C_{13}H_{22}N_2O_6S$ = 90 to 110†
Nicotinamide*	0.1	M.R.	12.21	$C_6H_6N_2O$ = (n.l.t.) 98.5
Nikethamide injection*	0.02	M.R.	3.565	$C_{10}H_{14}N_2O$ = 95 to 105†
Piperocaine hydrochloride	0.05	M.R.	14.89	$C_{16}H_{23}NO_2.HCl$ = (n.l.t.) 98
Piperocaine hydrochloride injection	0.05	M.R.	14.89	$C_{16}H_{23}NO_2.HCl$ = 95 to 105†
Procaine hydrochloride injection	0.05	M.R.	13.64	$C_{13}H_{20}N_2O_2.HCl$ = 95 to 105†
Procaine hydrochloride and epinephrine injection	0.05	M.R.	13.64	$C_{13}H_{20}N_2O_2.HCl$ = 95 to 105†
Quinine and urethane injection*	0.1	M.R.	8.909	$C_3H_7NO_2$ = 95 to 105†
Ringer's solution, lactated	0.1	M.O.	11.21	$C_3H_5NaO_3$ = 0.29 to 0.33 W/V

Table 15. Official Alkalimetric Assays by Residual Titration. (*Continued*)

Substance	Normality of acid	Indicator	Equivalent of 1 cc., mg.	Official requirement, per cent
U.S.P.				
Saccharin sodium tablets*	0.05	M.R.	12.06	$C_7H_4NNaO_3S.2H_2O$ = 95 to 110†
Sodium citrate	0.5	M.O.	43.02	$C_6H_5Na_3O_7$ = (n.l.t.) 99
Sodium citrate solution, anticoagulant	0.5	M.O.	49.02	$C_6H_5Na_3O_7.2H_2O$ = 2.35 to 2.65†
Sodium citrate solution, anticoagulant acid, dextrose	0.5	M.O.	49.02	$C_6H_5Na_3O_7.2H_2O$ = 95 to 105†
Sodium lactate injection	0.1	M.O.	11.21	$C_3H_5NaO_3$ = 95 to 110†
Sodium morrhuate injection	0.1	M.O.	32.40	Sodium morrhuate = 93 to 107†
Zinc oxide	1.0	M.O.	40.69	ZnO = (n.l.t.) 99
Zinc stearate	0.1	M.O.	4.069	ZnO = 13–15.5
N.F.				
Ammonia liniment*	1.0	M.R.	17.03	NH_3 = 2.0 to 2.8*W/V*
Ammonium acetate solution*	1.0	M.R.	77.08	CH_3COONH_4 = 6.5 to 7.5*W/V*
Ammonium valerate, acid*	0.1	M.R.	11.92	$C_4H_9COONH_4$ = 33 to 38
Beef extract*	0.1	M.R.	1.703	NH_4 = (n.l.t.) 0.35 of total solids
Bromides, three, tablets*	0.5	M.R.	48.98	NH_4Br = 30.8 to 35.8
Effervescent powders, compound	0.5	Pp.	42.01	$NaHCO_3$ = 23 to 27
		M.O.	70.56	$KNaC_4H_4O_6.4H_2O$ = 73 to 78
Ichthammol*	0.5	M.R.	8.516	NH_3 = (n.l.t.) 2.5
Iron and ammonium acetate solution*	0.5	M.R.	8.516	NH_3 = 0.6 to 0.8*W/V*
Methenamine and sodium biphosphate tablets*	0.5	M.R.	17.52	$C_6H_{12}N_4$ = 92.5 to 107.5†
Potassium acetate	0.5	M.O.	49.07	$C_2H_3KO_2$ = (n.l.t.) 99
Potassium citrate	0.5	M.O.	51.07	$C_6H_5K_3O_7$ = (n.l.t.) 99
Potassium sodium tartrate	0.5	M.O.	52.54	$C_4H_4KNaO_6$ = (n.l.t.) 99
Procaine hydrochloride tablets	0.1	M.R.	27.28	$C_{13}H_{20}N_2O_2.HCl$ = 92.5 to 107.5†
Silver nitrate solution, ammoniacal*	0.5	M.R.	8.516	NH_3 = 9.0 to 9.7
Sodium bicarbonate and calcium carbonate powder	1.0	Pp.	84.02	$NaHCO_3$ = 92.5 to 107.5†
Sodium bicarbonate and calcium carbonate tablets	1.0	Pp.	84.02	$NaHCO_3$ = 92.5 to 107.5†
Sodium bicarbonate and magnesium oxide powder	1.0	Pp.	84.02	$NaHCO_3$ = 92.5 to 107.5†
Sodium bicarbonate and magnesium oxide tablets	1.0	Pp.	84.02	$NaHCO_3$ = 92.5 to 107.5†
Zinc undecylenate	0.1	M.O.	21.60	$C_{22}H_{38}O_4Zn$ = 98 to 102

* Assay procedures in which distillation of ammonia or amine is involved.

† Per cent of labeled amount.

M.R. = methyl red, M.O. = methyl orange, Pp. = phenolphthalein.

(n.l.t.) = not less than.

tively liberated from an organic nitrogen containing compound by acid or alkaline hydrolysis as previously described. If the nitrogen of such a compound is in the trivalent state, amino or amido nitrogen, an equivalent amount of ammonia can be obtained as ammonium sulfate by digestion of the sample with concentrated sulfuric acid until decomposition is complete as indicated by the disappearance of carbon which is converted to carbon dioxide during the digestion. When the digestion is complete, the ammonia is liberated by treatment of the residue of ammonium sulfate and excess sulfuric acid with sodium hydroxide, and distilled into excess standard sulfuric acid or boric acid (1 in 25). The distillate in each case is titrated in the usual manner.

The time required for the digestion can be reduced by adding potassium sulfate in a sufficient amount so that the molal ratio of H_2SO_4 to K_2SO_4 is 1.5, thereby raising considerably the temperature at which the digestion is carried out (Gunning modification). The time required for the digestion can also be reduced by addition of catalysts such as copper sulfate or selenium.

If all or part of the nitrogen is present as nitrate, a further modification is necessary. Salicylic acid is added to the digestion mixture and, by its reaction with nitric acid liberated by the sulfuric acid, forms nitrosalicylic acid and prevents loss of the nitric acid along with other volatile substances formed during the digestion. After the mixture has stood with shaking at room tem-

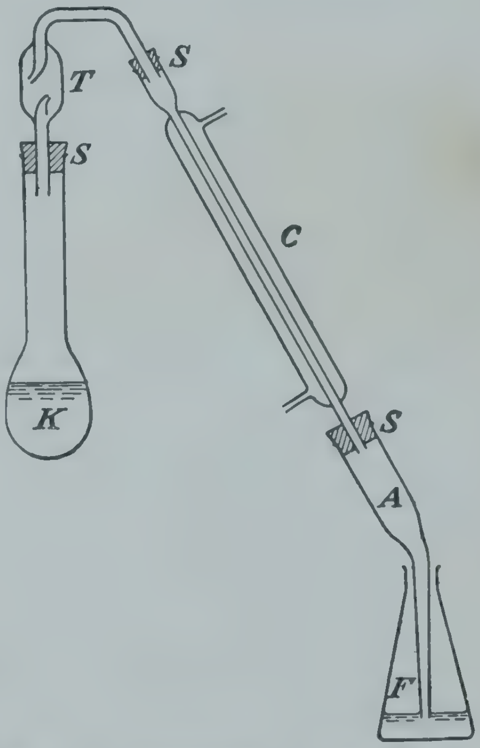


Fig. 14. Apparatus for Kjeldahl distillation. (*K*) 500-ml. Kjeldahl flask; (*S*) tightly fitting rubber stoppers; (*T*) spray trap or connecting bulb; (*C*) condenser; (*A*) adapter; (*F*) 500-ml. Erlenmeyer flask.

perature for 30 min., sodium thiosulfate is added, and the aminosalicylic acid formed by reduction of nitrosalicylic acid undergoes decomposition with formation of an equivalent amount of ammonia during the subsequent digestion.

In the U.S.P. and N.F., detailed directions for determination of total nitrogen are given. Method I is a macro method in which the ammonia is distilled into excess standard sulfuric acid, using the apparatus shown diagrammatically in Fig. 14. Method II is a semimicro method in which smaller samples are employed and the ammonia is distilled into boric acid using apparatus as described in the U.S.P.

Exercise 20

Object. Determination of the Nitrogen Content of the Alcohol-soluble Substances of Beef Extract.

Materials Required.
10 Gm. of beef extract.
100 cc. of alcohol.
50 cc. of $0.1N$ sulfuric acid.
50 cc. of $0.1N$ sodium hydroxide.
10 Gm. of powdered potassium sulfate.
20 cc. of sulfuric acid.

All the chemicals used must be of reagent quality and be free from nitrates and ammonium salts.

Procedure. 1. Weigh about 10 Gm. of beef extract accurately and add sufficient water to make 100 cc.

The beef extract can be weighed conveniently in a small evaporating dish and then washed into a 100-cc. volumetric flask with sufficient water to make 100 cc. of solution. The solution should be nearly clear and free from sediment to conform to N.F. specifications. This solution is used in the following steps:

2. "Distribute 10 cc. of the solution over clean, dry sand or asbestos, tared in a porcelain dish, and dry at 105° for 16 hours: the yield of residue is not less than 750 mg., equivalent to 75 per cent of total solids in the original Extract."

The solution of the extract is distributed over sand or asbestos to expose a large surface area and thereby facilitate evaporation of the water.

3. "Transfer 25 cc. of the solution to a 100-cc. Erlenmeyer flask, add 50 cc. of alcohol, and shake the mixture thoroughly. Collect the precipitate upon a counterpoised filter, wash it 3 times with a mixture of 2 volumes of alcohol and 1 volume of water, and dry at 105° for 2 hours: the weight of this precipitate, representing the alcohol-insoluble solids, does not exceed 10 per cent of the total solids in the solution taken. (*Reserve the filtrate and washings for the determination of nitrogen.*)"

The alcohol filtrate contains the alcohol-soluble substances of beef extract. This should be made up to a definite volume, so that an accurate aliquot portion may be measured in the next step.

4. "Measure an aliquot portion of the alcohol filtrate remaining from the test for *Alcohol-insoluble substances*, corresponding to 1 Gm. of the alcohol-soluble solids, into a 500-cc. Kjeldahl flask."

The aliquot portion corresponding to 1 Gm. of the alcohol-soluble solids of beef extract is calculated as follows: If the total solids, step 2, of a 10-cc. sample of the solution of the extract weighed 0.8000 Gm., then the total solids of a 25-cc. sample used in step 3 must have weighed 2.0000 Gm. If the alcohol-insoluble substances in this 25-cc. sample were found to weigh 0.2000 Gm., the 25-cc. sample must have contained $2.0000 - 0.2000 = 1.8000$ Gm. of alcohol-soluble substances. Therefore, $1/1.8 \times 25 = 13.9$ cc. of the 25-cc. sample of solution must have contained 1 Gm. of alcohol-soluble substances. If the filtrate containing the alcohol-soluble substances of the 25-cc. sample of solution measured 100 cc., then $13.9/25 \times 100 = 55.6$ cc. of the alcohol filtrate should be used, since it contains 1 Gm. of alcohol-soluble substances of beef extract.

5. "Add about 10 Gm. of powdered potassium sulfate and 20 cc. of sulfuric acid. Heat the mixture at a low temperature until frothing has ceased; then raise the temperature and boil the mixture until it acquires a pale yellow color, or is nearly colorless."

During this digestion, the organic components are decomposed to CO_2, H_2O, and NH_3. The latter remains in the residue as ammonium sulfate, while the CO_2 and H_2O are for the most part boiled off along with SO_2 resulting from reduction of sulfuric acid.

6. "Cool the flask, add about 250 cc. of water, and cautiously add a 30 per cent solution of sodium hydroxide until the contents of the flask are alkaline,

and then add 5 cc. more. Connect the flask at once by means of a spray trap to a condenser, the lower outlet tube of which dips beneath the surface of 50 cc. of 0.1*N* sulfuric acid, and distil the mixture until about 100 cc. of distillate has been collected in the acid."

Before adding the sodium hydroxide, a strip of litmus paper is dropped into the mixture and all connections, including the outlet tube of the condenser dipping into the standard acid, are made ready. A few pieces of granular zinc or porous plate, placed in the flask prior to distillation, will prevent bumping. The alkali liberates the ammonia from the ammonium sulfate, and upon distillation the ammonia gas is driven over and collected in the standard acid. In the analysis of compounds containing a high percentage of nitrogen, all the standard acid may be neutralized before the whole of the ammonia has distilled. If from 2 to 3 drops of methyl red are added to the standard acid before the distillation is started, the color of the indicator will show whether all the acid has been neutralized, and in case it has, more standard acid can be placed in the receiver without an appreciable loss of ammonia. The Kjeldahl trap in the apparatus prevents liquid from the distillation flask from being carried over mechanically.

7. "Titrate the excess acid with 0.1*N* sodium hydroxide, using methyl red T.S. as the indicator. Each cubic centimeter of 0.1*N* sulfuric acid is equivalent to 1.401 mg. of N. The amount of nitrogen thus found is not less than 60 mg."

Since 1 gram-atomic weight of nitrogen, 14.01 Gm., is necessary for formation of 1 mole of ammonia, the equivalent weight of nitrogen is 14.01 Gm. Each cubic centimeter of 0.1*N* sulfuric acid is equivalent to $0.1 \times 0.01401 = 0.001401$ Gm. or 0.1 meq. of nitrogen.

Calculate the number of milligrams of nitrogen in 1 Gm. of the alcohol-soluble substances of the beef extract and compare with the N.F. specifications.

The official alkalimetric assays by the macro method, Method I, are given in Table 16. The assay of methamphetamine hydrochloride for nitrogen content is the only official assay using the micro method, Method II. The macro method for this assay is also official, as an optional procedure.

The Kjeldahl method is applied widely in the analysis of proteins of food, fertilizer, blood, etc. Since the proportion of nitrogen in proteins is fairly constant, multiplication of the percentage of nitrogen found by a factor, 6.25, provides a rather accurate estimate of the proportion of protein in the sample.

TABLE 16. OFFICIAL ALKALIMETRIC ASSAYS BY THE MACRO KJELDAHL METHOD

Substance	Amount used, mg.	Official requirement, per cent*
U.S.P.		
Carbachol	200	N = 15.0 to 15.5
Methamphetamine hydrochloride	1,000†	N = 7.3 to 7.7
Yeast, dried	1,000	Protein = (n.l.t.) 40
Yeast, dried, tablets	1,000	Protein = (n.l.t.) 95
N.F.		
Aminoacetic acid	150	N = 18.4 to 18.8
Beef extract	1,000	N = (n.l.t.) 60 mg. per Gm. of alcohol-soluble substances
Histidine monohydrochloride	250	N = 21.5 to 22.2
Histidine monohydrochloride injection	250	N = 18.5 to 21.7

* 0.1*N* acid equivalent to 1.401 mg. of nitrogen is used in each.

† Also assayed by semimicro method.

(n.l.t.) = not less than.

Questions and Problems

1. What weight of each of the following should be used as sample in a semimicro Kjeldahl determination, so that 15 cc. of 0.01*N* sulfuric acid would be required in the titration: (*a*) histidine monohydrochloride; (*b*) carbachol; (*c*) sulfathiazole; (*d*) acetanilid?

2. Explain the purpose of each of the following in a Kjeldahl procedure: (*a*) potassium sulfate; (*b*) copper sulfate; (*c*) salicylic acid; (*d*) benzoic acid.

3. The ammonia from a 6.1500-Gm. sample of an organic nitrogen compound was distilled into 50.00 cc. of 0.1246*N* sulfuric acid and the mixture was titrated with 22.12 cc. of 0.0962*N* sodium hydroxide. Calculate the percentage of nitrogen in the sample.

4. A 2.5000-Gm. sample of acetanilid (98.5 per cent C_8H_9NO) was assayed by a Kjeldahl procedure. If the ammonia from the sample were distilled into 50.00 cc. of 1*N* sulfuric acid, what volume of 1.2450*N* sodium hydroxide would be required in the titration?

5. Calculate the per cent CH_4N_2O in a 0.5000-Gm. sample of urea, if 15.35 cc. of $0.4967N$ sodium hydroxide were required in the titration after the ammonia from the sample had been distilled into 50.00 cc. of $0.4861N$ sulfuric acid.

6. If the nitrogen content of the following is determined by a Kjeldahl procedure, what conversion factors would be used in calculation of the corresponding amount of the original compound: (*a*) histidine monohydrochloride; (*b*) aminoacetic acid?

Chapter 6

ACIDIMETRY

Acids are estimated quantitatively by methods analogous to those employed in alkalimetry, *viz.*, by directly titrating an exact quantity of the acid, acid salt, or other acidic substance with standard alkali solutions or by adding an excess of the latter and determining the amount in excess by residual titration with standard acid solution. Direct titration is employed whenever practicable, since it is easier and requires fewer measurements of volume.

In assaying acids, the quantity of acid to be taken should be such that about 30 to 40 cc. of the alkali solution will be consumed. As a general principle, it is recommended that the normality of the acid to be titrated should be approximately the same as that of the titrating medium. Except when otherwise directed, the liquid to be titrated should be brought to room temperature before titration, as many indicators give different values at different temperatures. For most of the inorganic acids, methyl orange, methyl red, or phenolphthalein can be used as indicators, and the alkali should be standardized with the particular indicator used. For organic acids, phenolphthalein is almost always used; however, thymol blue or bromothymol blue may be employed in some assays.

DIRECT TITRATION METHODS

Exercise 21

Object. Assay of Hydrochloric Acid.

Materials Required. 3 cc. of hydrochloric acid.

1*N* sodium hydroxide.

Procedure. "Tare a glass-stoppered flask containing about 20 cc. of water, add about 3 cc. of Hydrochloric Acid, and reweigh. Dilute with about 25 cc. of water and titrate with 1*N* sodium hydroxide, using methyl red T.S. as the indicator. Each cubic centimeter of 1*N* sodium hydroxide is equivalent to 36.47 mg. of HCl."

Concentrated acid solutions are weighed in glass-stoppered flasks to prevent loss of dissolved gases, in some cases, and to prevent the absorption of water, in other cases. Samples are taken by weight rather than by volume, because it is difficult to measure small volumes of concentrated acids accurately, and a small error in the measurement of the sample would cause a large per cent error in the result.

$$\underset{36.47}{HCl} + NaOH \longrightarrow NaCl + H_2O$$

The equivalent weight of HCl is 36.47 Gm. Consequently each cubic centimeter of $1N$ sodium hydroxide is equivalent to 0.03647 Gm., 1 meq., of HCl, and the proportion of HCl in the sample is

$$\frac{\text{Volume of } 1N \text{ sodium hydroxide} \times 0.03647 \times 100}{\text{Wt. of sample}} = \text{per cent, } W/W$$

Questions and Problems

1. On the basis of the assay procedures, why is the concentration of hydrochloric acid, U.S.P., expressed as per cent *W/W* while that of diluted hydrochloric acid is expressed as per cent *W/V*?

2. Refer to the U.S.P. monographs for necessary data and calculate the per cent *W/V* concentration of hydrochloric acid, U.S.P., and the per cent *W/W* concentration of diluted hydrochloric acid, U.S.P.

3. A 4.0520-Gm. sample of hydrochloric acid, sp. gr. 1.18, required 44.15 cc. of $0.9035N$ sodium hydroxide in a titration. Calculate (*a*) HCl content in per cent; (*b*) normality; (*c*) Na_2CO_3 titer of the sample.

4. If 25.00 cc. of a hydrochloric acid solution required 26.25 cc. of $0.0952N$ sodium hydroxide in a titration, what volume of the solution would produce 0.2867 Gm. of AgCl?

5. A hydrochloric acid solution has an AgCl titer of 0.1265; what is its Na_2CO_3 titer?

Exercise 22

Object. Assay of Diluted Sulfuric Acid.

Materials Required. 10 cc. of diluted sulfuric acid.
$1N$ sodium hydroxide.

Procedure. "Accurately measure 10 cc. of Diluted Sulfuric Acid, and dilute with about 20 cc. of water. Titrate the solution with $1N$ sodium hydroxide, using methyl red T.S. as the indicator. Each cubic centimeter of 1N sodium hydroxide is equivalent to 49.04 mg. of H_2SO_4."

The official diluted acids are assayed, as a rule, by measuring an exact quantity of the acid, titrating it after dilution, and calculating the per cent of acid on a weight-to-volume basis. This method of sampling is more convenient and rapid than the taking of weighed samples, and since the volume of diluted acid used as sample is relatively large, the error in measuring is slight. The sulfuric acid reacts with the sodium hydroxide as indicated by the following equation:

$$\underset{98.08}{H_2SO_4} + 2NaOH \longrightarrow Na_2SO_4 + 2H_2O$$

Sulfuric acid is a dibasic acid and its equivalent weight is one-half its gram-molecular weight, or 49.04 Gm. Therefore, each cubic centimeter of 1N sodium hydroxide is equivalent to 49.04 mg. or 1 meq. of H_2SO_4. Calculate the H_2SO_4 content of the sample in per cent W/V.

Questions and Problems

1. Hypophosphorous acid, U.S.P., contains about 31 per cent HPH_2O_2 and nitric acid, N.F., contains about 69 per cent HNO_3. Both are assayed by titration with normal sodium hydroxide to a methyl red end point. What weight of each would be used as sample in order that 35 cc. of 1N sodium hydroxide would be consumed in the titration? Compare answers with the weights suggested in the monographs.

2. A 10.00-cc. sample of sulfuric acid solution required 16.85 cc. of a sodium hydroxide solution in a titration. Each cubic centimeter of the sodium hydroxide solution was equivalent to 0.2472 Gm. of pure potassium biphthalate. Calculate the H_2SO_4 content of the sulfuric acid solution in per cent W/V.

3. Calculate the $BaSO_4$ and Na_2CO_3 titers of the sulfuric acid solution described in problem 2.

Exercise 23

Object. Assay of Boric Acid.

Materials Required. 2 Gm. of boric acid.
100 cc. of glycerin.
1N sodium hydroxide.

Procedure. "Dry about 2 Gm. of Boric Acid over sulfuric acid for 5 hours, weigh accurately, and dissolve the dried Acid in 100 cc. of a mixture of equal volumes of glycerin and water, previously neutralized to phenolphthalein T.S. Titrate with 1*N* sodium hydroxide, using phenolphthalein T.S. as the indicator. Discharge the pink color by the addition of 50 cc. of glycerin, neutralized to phenolphthalein T.S., and continue the titration until the pink color reappears. Each cubic centimeter of 1*N* sodium hydroxide is equivalent to 61.84 mg. of H_3BO_3."

Boric acid behaves as a very weak monobasic acid,

$$H_3BO_3 \rightleftharpoons H^+ + BO_2^- + H_2O$$

because the metaborate ion is strongly basic and forces the above reaction to the left. Long before an equivalent amount of sodium hydroxide has been added, the BO_2^- ion concentration is sufficiently high and the H^+ ion concentration consequently sufficiently low that a change in the color of the phenolphthalein indicator occurs. If glycerin is added, however, it forms a slightly dissociated complex with the metaborate ion and thus prevents reversal of the reaction. As a result, the pH during the titration remains below 8.0 until an equivalent of sodium hydroxide has been added. In other words, in the presence of glycerin, boric acid behaves like a sufficiently strong monobasic acid that its titration with standard alkali is feasible when phenolphthalein is used as indicator.

Since H_3BO_3 behaves as a monobasic acid in this titration, 1 mole is 1 equivalent and the equivalent weight is 61.84 Gm. Each cubic centimeter of 1*N* sodium hydroxide is equivalent to 61.84 mg. or 1 meq. of H_3BO_3.

Questions and Problems

1. What preliminary treatment is necessary before the H_3BO_3 in boric acid ointment can be determined titrimetrically?

2. In the assay of boric acid ointment, about 10 per cent H_3BO_3, an aliquot part corresponding to one-fifth of the original sample is titrated in the usual way with 0.1*N* sodium hydroxide. Explain why an original sample weighing 5 Gm. is employed.

3. In the assay of boroglycerin glycerite, 1*N* sodium hydroxide is used. Calculate the $C_3H_5BO_3$ titer of the standard solution and the weight of boroglycerin glycerite, about 50 per cent $C_3H_5BO_3$, that would be used as sample so that 35 cc. of 1*N* sodium hydroxide would be consumed. Compare answers with the quantities given in the N.F. monograph.

Exercise 24

Object. Assay of Phenyl Salicylate Tablets.

Materials Required. 20 phenyl salicylate tablets.
30 cc. of sodium hydroxide T.S.
Hydrochloric acid T.S.
Ether.
Neutralized alcohol.
0.1*N* sodium hydroxide.

Procedure. 1. "Weigh a counted number of not less than 20 Phenyl Salicylate Tablets, reduce them to a fine powder without an appreciable loss, and heat an accurately weighed portion, equivalent to about 300 mg. of phenyl salicylate, with 30 cc. of sodium hydroxide T.S., for 45 minutes on a water bath."

An accurate weight of a counted number of tablets, before powdering, is necessary so that the amount of phenyl salicylate in each tablet can be computed from the analytical data. If twenty 5-grain tablets were used, they would be presumed to contain 20 × 5 × 65 = 6,500 mg. of phenyl salicylate. If the combined weight of the 20 tablets were 8.5800 Gm., then 300/6,500 × 8.5800 = 0.395 Gm. of the powdered tablets should contain about 300 mg. of phenyl salicylate. Therefore a sample of the powdered tablets weighing about 400 mg. is used.

The phenyl salicylate is hydrolyzed to sodium salicylate and phenol by heating the sample with sodium hydroxide T.S.:

$$\underset{214.21}{C_{13}H_{10}O_3} + NaOH \longrightarrow C_7H_5NaO_3 + C_6H_6O$$

The phenolic hydroxyls of both phenol and salicylic acid react with sodium hydroxide at high pH, but this reaction need not be considered as far as stoichiometry in this assay is concerned.

2. "Transfer the mixture to a separator, acidify it with hydrochloric acid T.S., and completely extract the salicylic acid with successive portions of ether. Wash the combined ether solutions with 10 cc. of water; then evaporate the ether at a low temperature, allowing the last few cubic centimeters to evaporate spontaneously."

Both salicylic acid and phenol are liberated when the mixture is acidified, and the salicylic acid is extracted completely with ether.

A considerable proportion of the phenol also dissolves in the ether but phenol is such a weak acid that it will not interfere in the subsequent titration of the salicylic acid. The evaporation must be conducted cautiously to avoid decarboxylation of the salicylic acid or loss by sublimation.

3. "Dissolve the residue in a few cubic centimeters of neutralized alcohol, add 15 cc. of water, and titrate with 0.1*N* sodium hydroxide, using phenolphthalein T.S. as the indicator. Each cubic centimeter of 0.1*N* sodium hydroxide is equivalent to 21.42 mg. of $C_{13}H_{10}O_3$."

Each mole of phenyl salicylate, on hydrolysis, produces 1 mole of salicylic acid, a monobasic acid. Therefore the equivalent weight of phenyl salicylate is 214.21 Gm.; and each cubic centimeter of 0.1*N* sodium hydroxide, 0.1 meq., is equivalent to $0.1 \times 0.21421 = 0.021421$ Gm. or 0.1 meq. of phenyl salicylate.

Questions and Problems

1. Explain why none of the standard sodium hydroxide used in the assay of phenyl salicylate tablets is neutralized by reaction with the phenolic hydroxyls of salicylic acid and phenol.

2. Compare the procedures used for extraction of salicylic acid in phenyl salicylate tablets, N.F., and mandelic acid in Ammonium mandelate tablets, U.S.P., and explain any difference.

3. If twenty 5-grain phenyl salicylate tablets weighed 9.1246 Gm., what weight of the powdered tablets would be used in the analysis?

4. If phenyl salicylate tablets contained 102.5 per cent of the labeled amount, 5 grains, of phenyl salicylate ($C_{13}H_{10}O_3$) and 20 tablets weighed 9.1246 Gm., what volume of 0.0945*N* sodium hydroxide would be required in titration of the salicylic acid obtained from 0.4165 Gm. of the powdered tablets?

5. Calculate the limits of the weight of phenyl salicylate that a phenyl salicylate tablet might contain and still conform to the N.F. specifications.

Exercise 25

Object. Assay of Tartaric Acid.

Materials Required. 3 Gm. of tartaric acid.
1*N* sodium hydroxide.

Procedure. "Place about 2 Gm. of Tartaric Acid, previously dried over sulfuric acid for 3 hours and accurately weighed, in an Erlenmeyer flask. Dissolve it in 40 cc. of water, and titrate with 1*N* sodium hydroxide, using phenolphthalein T.S. as the indicator. Each cubic centimeter of 1*N* sodium hydroxide is equivalent to 75.04 mg. of $C_4H_6O_6$."

Phenolphthalein is the most suitable indicator to use in the titration of organic acids. Tartaric acid reacts as a dibasic acid as follows:

$$\underset{150.09}{C_2H_2(OH)_2(COOH)_2} + 2NaOH \longrightarrow Na_2C_4H_4O_6 + 2H_2O$$

As indicated by the equation, 2 moles of sodium hydroxide are required to neutralize 1 mole of tartaric acid; hence the equivalent weight of tartaric acid is 75.04 Gm. Each cubic centimeter of 1*N* sodium hydroxide is equivalent to 75.04 mg. or 1 meq. of tartaric acid.

In Table 17 are listed a large number of organic acids which are assayed by a direct alkalimetric procedure. 0.1*N* or 1*N* sodium hydroxide is generally used; however, 0.1*N* potassium hydroxide is specified in the official procedure for undecylenic acid. In most titrations, phenolphthalein is employed as indicater. Bromothymol blue, changing at pH 6.0 to 7.6, is employed in some procedures, including those for glutamic acid hydrochloride and propylthiouracil. It should also be noted that phosphoric acid behaves as a dibasic acid when titrated to a phenolphthalein end point because the ionization constant, 5×10^{-13}, for the third proton is very small. The $HPO_4^=$ ion is very weakly acidic and is not neutralized at the pH where phenolphthalein changes color. As a result, the equivalent weight of H_3PO_4 is 49.00 Gm., ½ mole, and that of $NaH_2PO_4 \cdot H_2O$ is 138.01 Gm., 1 mole.

The formol titration procedure of Sörensen is employed for the determination of the aminoacetic acid content of aminoacetic acid elixir. An aliquot part of a decolorized and filtered solution of a sample of the elixir is titrated to a phenolphthalein end point with 0.1*N* sodium hydroxide. An equal aliquot part is treated with formaldehyde T.S. and titrated to a phenolphthalein end point with 0.1*N* sodium hydroxide and a blank is run on the formaldehyde T.S. Only in the presence of formaldehyde is the aminoacetic acid neutralized by the standard sodium hydroxide on titration to a phenolphthalein end point, because the formaldehyde reacts with the amino group forming $H_2C{=}NCH_2COOH$ and prevents the intramolecular reaction of the amino and carboxyl groups which produces

TABLE 17. OFFICIAL ACIDIMETRIC ASSAYS BY DIRECT TITRATION

Substance	Normality of alkali	Indicator	Equivalent of 1 cc., mg.	Official requirement, per cent
U.S.P.				
Acetic acid	1.0	Pp.	60.05	$C_2H_4O_2$ = 36 to 37
Acetic acid, glacial	1.0	Pp.	60.05	$C_2H_4O_2$ = (n.l.t.) 99.4
Ammonium mandelate tablets	0.1	Pp.	16.92	$C_8H_7O_3NH_4$ = 93 to 107*
Benzoic acid	0.1	Pp.	12.21	$C_7H_6O_2$ = (n.l.t.) 99.3
Boric acid	1.0	Pp.	61.84	H_3BO_3 = (n.l.t.) 99.5
Boric acid ointment	0.1	Pp.	6.184	H_3BO_3 = 9 to 11
Calcium mandelate tablets	0.1	Pp.	17.12	$C_{16}H_{14}CaO_6$ = 95 to 105*
Citric acid	1.0	Pp.	70.05	$C_6H_8O_7.H_2O$ = (n.l.t.) 99.7
Hydrochloric acid	1.0	M.R.	36.47	HCl = 35 to 38
Hydrochloric acid, diluted	1.0	M.R.	36.47	HCl = 9.5 to 10.5 *W/V*
Hypophosphorous acid	1.0	M.R.	66.00	HPH_2O_2 = 30 to 32
Iodoalphionic acid tablets	0.1	T.B.	24.70	$C_{15}H_{12}I_2O_3$ = 94 to 106*
Nicotinic acid	0.1	Pp.	12.31	$C_6H_5NO_2$ = (n.l.t.) 99.5
Propylthiouracil	0.1	B.T.B.	8.512	$C_7H_{10}N_2OS$ = (n.l.t.) 98
Propylthiouracil tablets	0.1	B.T.B.	8.512	$C_7H_{10}N_2OS$ = 93 to 107*
Saccharin	0.1	Pp.	18.32	$C_7H_5NO_3S$ = (n.l.t.) 98
Saccharin sodium	0.1	Pp.	20.52	$C_7H_4NNaO_3S$ = (n.l.t.) 98
Salicylic acid	0.1	Pp.	13.81	$C_7H_6O_3$ = (n.l.t.) 99.5
Sodium biphosphate	1.0	Pp.	138.0	$NaH_2PO_4.H_2O$ = 98 to 103
Sodium citrate solution, anticoagulant acid, dextrose	0.1	Pp.	7.005	$C_6H_8O_7.H_2O$ = 95 to 105*
Tartaric acid	1.0	Pp.	75.04	$C_4H_6O_6$ = (n.l.t.) 99.7
Trichloroacetic acid	1.0	Pp.	163.4	$C_2HCl_3O_2$ = (n.l.t.) 99
N.F.				
Acetic acid, diluted	1.0	Pp.	60.05	$C_2H_4O_2$ = 5.7 to 6.3 *W/V*
Acetophenetidin and phenyl salicylate tablets	0.1	Pp.	21.42	$G_{13}H_{10}O_3$ = 90 to 110*
Aminoacetic acid elixir	0.1	Pp.	7.507	$C_2H_5NO_2$ = 12.1 to 14.2 *W/V*
Ammonium valerate, acid	0.1	Pp.	10.21	C_4H_9COOH = 62 to 67
Boric acid solution	1.0	Pp.	61.84	H_3BO_3 = (n.l.t.) 4.25
Boroglycerin glycerite	1.0	Pp.	99.89	$C_3H_5BO_3$ = 47.5 to 52.5
Caffeine, citrated	0.1	Pp.	6.404	$C_6H_8O_7$ = 48 to 52
Cinchophen	0.1	Pp.	24.93	$C_{16}H_{11}NO_2$ = (n.l.t.) 99.5
Cinchophen tablets	0.1	Pp.	24.93	$C_{16}H_{11}NO_2$ = 92.5 to 107.5*
Dehydrocholic acid	0.1	Pp.	40.25	$C_{24}H_{34}O_5$ = 98.5 to 101.5 (oral), 99 to 101 (parenteral)
Dehydrocholic acid tablets	0.1	Pp.	40.25	$C_{24}H_{34}O_5$ = 92.5 to 107.5*
Effervescent powders, compound	1.0	Pp.	75.05	$C_4H_4O_6$ = 2 to 2.4
Formic acid	0.1	Pp.	4.603	CH_2O_2 = 24 to 26
Glutamic acid hydrochloride	0.1	B.T.B.	9.180	$C_5H_9NO_4.HCl$ = 99 to 101
Glutamic acid hydrochloride capsules	0.1	B.T.B.	9.180	$C_5H_9NO_4.HCl$ = 93 to 107*
Mandelic acid	0.1	Pp.	15.21	$C_8H_8O_3$ = (n.l.t.) 99
Nitric acid	1.0	M.R.	63.02	HNO_3 = 67 to 71
Phenylsalicylate tablets	0.1	Pp.	21.42	$C_{13}H_{10}O_3$ = 91 to 109*
Phosphoric acid	1.0	Pp.	49.00	H_3PO_4 = 85 to 88
Phosphoric acid, diluted	1.0	Pp.	49.00	H_3PO_4 = 9.5 to 10.5 *W/V*
Potassium bitartrate	1.0	Pp.	188.2	$C_4H_5KO_6$ = (n.l.t.) 99.5
Sodium dehydrocholate injection	0.1	Pp.	42.45	$C_{24}H_{33}NaO_5$ = 94 to 106*
Sodium salicylate and iodide with colchicine injection	0.1	Pp.	16.01	$C_7H_5NaO_3$ = 93 to 103*
Sulfuric acid	1.0	M.R.	49.04	H_2SO_4 = 94 to 98
Sulfuric acid, diluted	1.0	M.R.	49.04	H_2SO_4 = 9.5 to 10.5 *W/V*
Theobromine and sodium salicylate	0.1	Pp.	13.81	$C_7H_6O_3$ = (n.l.t.) 35
Undecylenic acid	0.1	Pp.	18.43	$C_{11}H_{20}O_2$ = (n.l.t.) 95

* Per cent of labeled amount.

Pp. = phenolphthalein, M.R. = methyl red, T.B. = thymol blue, B.T.B. = bromothymol blue.

(n.l.t.) = not less than.

the very weakly acidic Zwitterion, $H_3^+N—CH_2COO^-$. The formaldehyde derivative neutralizes sodium hydroxide like any other carboxylic acid. The difference between the volume of 0.1*N* sodium hydroxide consumed by the aliquot part of the sample treated with formaldehyde, the volume being corrected in accordance with the blank, and the volume of sodium hydroxide consumed by the untreated aliquot part is a measure of the aminoacetic acid present in the aliquot part.

Questions and Problems

1. Why is bromothymol blue used as indicator in the assay of glutamic acid hydrochloride, while phenolphthalein is used in the assay of tartaric acid? Write equations for the reactions involved.

2. Calculate the following titer values for 0.1*N* sodium hydroxide and compare the calculated values with those given in the official monographs: (*a*) salicylic acid, $C_7H_6O_3$; (*b*) iodoalphionic acid, $C_{15}H_{12}I_2O_3$; (*c*) nicotinic acid, $C_6H_5NO_2$.

3. Calculate the equivalent weights of three acids assayed by use of 0.1*N* sodium hydroxide and of three acids assayed by use of 1*N* sodium hydroxide. Suggest a reason for the choice of 1*N* or 0.1*N* solutions in acidimetry.

4. What weight of each of the following substances would be used as sample so that 35 cc. of 1*N* sodium hydroxide would be consumed in titration to a phenolphthalein end point? (*a*) Phosphoric acid, H_3PO_4; (*b*) sodium biphosphate, $NaH_2PO_4.H_2O$; (*c*) citric acid, $C_6H_8O_7.H_2O$; (*d*) tartaric acid, $C_4H_6O_6$. Compare answers with sample weights specified in the official monographs.

5. Write equations for all reactions involved in the assay of aminoacetic acid elixir. What is Sörensen's "formol titration"?

RESIDUAL TITRATION METHODS

Exercise 26

Object. Assay of Acetylsalicylic Acid Tablets.

Materials Required. 20 acetylsalicylic acid tablets. (Note: Two 5-grain tablets may be used for student work provided that the instructor gives the weight of 20 tablets.)

0.1*N* sulfuric acid.

0.1*N* sodium hydroxide.

Procedure. 1. "Weigh a counted number of not less than 20 Acetylsalicylic Acid Tablets, and reduce them to a fine powder without appreciable loss. Weigh accurately a portion of the powder, equivalent to about 500 mg. of acetylsalicylic acid, transfer it completely to an Erlenmeyer flask with the aid of 20 cc. of neutralized alcohol, previously cooled to from 15° to 20°, and titrate the solution immediately with 0.1*N* sodium hydroxide, using phenolphthalein T.S. as the indicator."

Care must be observed in powdering the tablets because the sample will not be representative if any of the material is lost. The sample is titrated with sodium hydroxide to neutralize any free acid formed by hydrolysis of the acetylsalicylic acid as represented by the equation:

$$C_6H_4.OCOCH_3.COOH + HOH \longrightarrow C_6H_4.OH.COOH + CH_3COOH$$

and to neutralize the carboxy group of the acetylsalicylic acid:

$$\underset{180.15}{C_6H_4.OCOCH_3.COOH} + NaOH \longrightarrow C_6H_4.OCOCH_3COONa + HOH$$

2. "Add to the titrated solution a volume of 0.1*N* sodium hydroxide equal to that used in the titration, plus 15 cc. more, and heat the mixture in a bath of boiling water for 15 minutes with frequent agitation. Remove the flask from the water bath, cool it quickly to room temperature, and titrate with 0.1*N* sulfuric acid. Perform a blank determination with the same quantities of the same reagents and in the same manner, and make any necessary correction. Subtract the number of cubic centimeters of 0.1*N* sulfuric acid used from the number of cubic centimeters of 0.1*N* sodium hydroxide added the second time, and multiply the difference by 18.02. The result represents the milligrams of $C_9H_8O_4$ present in the portion of the tablets used for the assay."

After the acetylsalicylic acid and its hydrolysis products, acetic acid and salicylic acid, have been neutralized in the first titration with 0.1*N* sodium hydroxide carried out at room temperature or slightly below, the only further reaction of the sample with the 0.1*N* sodium hydroxide added in excess to the titrated mixture involves saponification of the sodium acetylsalicylate as follows:

$$C_6H_4.OCOCH_3.COONa + NaOH \longrightarrow C_6H_4.OH.COONa + CH_3COONa$$

Consequently each cubic centimeter of 0.1*N* sodium hydroxide consumed in this reaction is equivalent to 0.1 meq. of sodium acetylsalicylate and, in turn, to 0.1 meq. of acetylsalicylic acid; *i.e.*, $0.1 \times 0.18015 = 0.018015$ Gm. of acetylsalicylic acid.

The acetylsalicylic acid content of the sample employed should be calculated and, from that, the amount in each tablet and percentage of the labeled amount.

Table 18. Official Acidimetric Assays by Residual Titration

Substance	Normality of alkali	Indicator	Equivalent of 1 cc., mg.	Official requirement, per cent
U.S.P.				
Acetylsalicylic acid	0.5	Pp.	45.04	$C_9H_8O_4$ = (n.l.t.) 99.5
Acetylsalicylic acid capsules	0.1	Pp.	18.02	$C_9H_8O_4$ = 93 to 107*
Acetylsalicylic acid tablets	0.1	Pp.	18.02	$C_9H_8O_4$ = 95 to 105*
Aluminum acetate solution	0.5	Pp.	30.03	$C_2H_4O_2$ = 4.24 to 5.11 W/V
Aluminum phosphate gel	0.5	Pp.	2.651	$AlPO_4$ = 3.8 to 4.5
Aluminum subacetate solution	0.5	Pp.	30.03	$C_2H_4O_2$ = 5.43 to 6.13 W/V
Benzyl benzoate	0.5	Pp.	106.1	$C_{14}H_{12}O_2$ = (n.l.t.) 99
Benzyl benzoate lotion	0.5	Pp.	106.1	$C_{14}H_{12}O_2$ = 26 to 30
Chloral hydrate	1.0	Pp.	165.4	$C_2H_3Cl_3O_2$ = (n.l.t.) 99.5
Dimethyl phthalate	0.5	T.B.	48.55	$C_{10}H_{10}O_4$ = (n.l.t.) 98
Glyceryl triacetate	0.5	Pp.	36.37	$C_9H_{14}O_6$ = (n.l.t.) 98.5
Hydriodic acid, diluted	0.5	Pp.	1.435	HPH_2O_2 = 0.6 to 1.0 W/V
Lactic acid	1.0	Pp.	90.08	$C_3H_6O_3$ = 85 to 90
Methylparaben	1.0	B.T.B.	152.1	$C_8H_8O_3$ = (n.l.t.) 99
Neocinchophen tablets	0.1	Pp.	29.13	$C_{19}H_{17}NO_2$ = 94 to 106*
Propylparaben	1.0	B.T.B.	180.2	$C_{10}H_{12}O_3$ = (n.l.t.) 99
Sodium phosphate	1.0	Pp.	6.180	Na_2HPO_4 = (n.l.t.) 98
Sodium phosphate, exsiccated	1.0	Pp.	6.180	Na_2HPO_4 = (n.l.t.) 98
Trimethadione	0.1	C.R.	14.31	$C_6H_9NO_3$ = (n.l.t.) 98
Trimethadione capsules	0.1	C.R.	14.31	$C_6H_9NO_3$ = 94 to 106*
Trimethadione tablets	0.1	C.R.	14.31	$C_6H_9NO_3$ = 94 to 106*
N.F.				
Alcohol rubbing compound	0.1	Pp.	8.482	$C_{28}H_{38}O_{19}$ = (n.l.t.) 0.355 W/V
Benzyl benzoate, saponated	0.5	Pp.	106.1	$C_{14}H_{12}O_2$ = 93 to 107 W/V
Calcium phosphate, tribasic	1.0	Pp.	6.743	$Ca_3(PO_4)_2$ = (n.l.t.) 90
Ethyl acetate	0.5	Pp.	44.05	$C_4H_8O_2$ = (n.l.t.) 99
Formaldehyde solution	1.0	B.T.B.	30.03	CH_2O = (n.l.t.) 37
Formic acid spirit	0.1	Pp.	4.603	CH_2O_2 = 0.95 to 1.05 W/V
Magnesium phosphate, tribasic	1.0	Pp.	5.716	$Mg_3(PO_4)_2$ = (n.l.t.) 98
Magnesium phosphate, tribasic, tablets	1.0	Pp.	7.674	$Mg_3(PO_4)_2.5H_2O$ = 93 to 107*
Methenamine and sodium biphosphate tablets	0.5	Pp.	3.000	$NaH_2PO_4.H_2O$ = 92.5 to 107.5*

* Per cent of labeled amount.

Pp. = phenolphthalein, T.B. = thymol blue, B.T.B. = bromothymol blue, C.R. = cresol red.

(n.l.t.) = not less than.

A number of official esters and preparations thereof are assayed by a residual acidimetric procedure, *e.g.*, ethyl acetate, glyceryl triacetate, dimethyl phthalate, neocinchophen tablets, etc. Other organic substances which react slowly with sodium hydroxide and which are assayed by residual methods include trimethadione, chloral hydrate, lactic acid, formaldehyde solution, etc. The acetic acid in aluminum acetate solution and aluminum subacetate solution is distilled into a measured excess of standard alkali and the distillate is titrated with standard acid, a procedure analogous to that used in the assay of preparations containing ammonia, ammonium salts, etc.

The ammonium phosphomolybdate, $(NH_4)_3PO_4.12MoO_3.H_2O$, produced quantitatively from various phosphates and hypophosphorous acid, is measured by a residual acidimetric procedure according to the following equation:

$$(NH_4)_3PO_4.12MoO_3.H_2O + 23NaOH \longrightarrow 11Na_2MoO_4 + (NH_4)_2MoO_4 + NaNH_4HPO_4 + 12H_2O$$

A list of official substances assayed by residual acidimetric procedures appears in Table 18.

Questions and Problems

1. If 0.6000 Gm. of the powder obtained by grinding twenty 5-grain aspirin tablets weighing 9.0000 Gm. were found to contain 0.4800 Gm. of acetylsalicylic acid, what was the percentage of the labeled amount (5 grains) of acetylsalicylic acid in the tablets?

2. Twenty 5-grain aspirin tablets weighed 8.5000 Gm. A 0.5600-Gm. sample of the powdered tablets was titrated at 20° to a phenolphthalein end point with $0.1N$ sodium hydroxide, treated with 40.00 cc. of $0.1N$ sodium hydroxide and, after heating on a water bath for 15 min., was titrated again to a phenolphthalein end point with 15.00 cc. of $0.1N$ sulfuric acid. No correction for the blank was necessary. Calculate the acetylsalicylic acid content of the tablets and express as a percentage of the labeled amount.

3. Calculate the weight of each of the following that is equivalent to 1 cc. of $0.5N$ alcoholic potassium hydroxide and check values with those given in the official monographs: (*a*) glyceryl triacetate; (*b*) dimethyl phthalate; (*c*) benzyl benzoate.

4. Write equations for all reactions involved in the assay of sodium phosphate and calculate the equivalent weight of Na_2HPO_4. What is the Na_2HPO_4 titer of $0.5N$ sodium hydroxide?

Chapter 7

PRECIPITATION METHODS

Under Neutralization Methods, a class of reactions was considered that were of value in quantitative analysis because very slightly ionized substances or gases or both were formed. In the following exercises, a class of reactions is dealt with that requires the formation of very slightly soluble substances to cause the reactions to go to sufficient completion to be quantitative in nature. The solubility product principle may be applied to all precipitation reactions, and is should be reviewed before the subsequent assays are undertaken.

Determination of the End Point. The end point of a reaction in analyses by precipitation methods may be determined in one of three ways:

1. By adding a standard solution to a solution of the substance being analyzed until no further precipitate is produced. This method is sometimes applied in the determination of the chloride ion content of chlorides with standard solution of silver nitrate.

2 By adding the standard solution to a clear solution of the substance being analyzed until a precipitate begins to form. This method is often used in the titration of alkali cyanides with standard silver nitrate solution. (See sodium cyanide reagent, N.F.)

3. By means of a suitable indicator. All the official assays by precipitation methods employ indicator solutions to show the end point of the reaction.

Indicators. The indicators used in the official volumetric precipitation assays are:

1. *Ferric ammonium sulfate T.S.*, prepared by dissolving 8 Gm. of reagent ferric ammonium sulfate in sufficient distilled water to make 100 cc. This indicator is used in both direct and residual titrations of silver and mercury salts with standard ammonium thiocyanate solution. The thiocyanate reacts with the Ag^+ or Hg^{++} present to form a white precipitate of silver or mercury thiocyanate, but as

soon as all the Ag^+ or Hg^{++} has been precipitated, the thiocyanate reacts with ferric ammonium sulfate to form red ferric thiocyanate; the first appearance of the red color marks the end point of the reaction.

2. *Potassium chromate T.S.*, prepared by dissolving 10 Gm. of reagent potassium chromate in sufficient distilled water to make 100 cc. It may be used as indicator in the titration of chloride solutions with standard silver nitrate solution, but in the National Formulary it is employed only in the assay of bitter almond oil for hydrocyanic acid. The end point is shown by the production of a permanent red color caused by the formation of silver chromate.

3. *Dichlorofluorescein T.S.*, prepared by dissolving 0.1 Gm. of dichlorofluorescein in 60 cc. of alcohol, adding 2.5 cc. of $0.1N$ sodium hydroxide, mixing and diluting to 100 cc. with water. Dichlorofluorescein T.S. is used as an adsorption indicator in the direct titration of soluble halides, with standard silver nitrate. A color change does not take place during the titration until Ag^+ is in excess, at which time the colloidal silver halide particles bear a positive charge because of a primary adsorption layer of silver cations. A secondary adsorption of the dichlorofluorescein anions results in a change in the electron distribution of the anions and a concomitant change in color. Before the end point is reached, halide anions are primarily adsorbed and the resulting negatively charged particles repel the fluorescein anion.

4. *Diiodofluorescein T.S.*, prepared by dissolving 0.5 Gm. of diiodofluorescein in a mixture of 75 cc. of alcohol and 30 cc. of water. It is used like dichlorofluorescein T.S. in the direct titration of halides with silver nitrate.

5. *Starch T.S.*, prepared as directed on page 187. Starch T.S. is used as indicator in the assay of iodine ointment and in the direct titration of iodide produced by oxidative degradation of various organic iodine compounds with standard silver nitrate. The blue color of the starch-iodide-iodine complex persists until the iodide ion has been consumed by silver nitrate in the titration.

Standard Solutions. The standard solutions employed in the official assays by precipitation methods are $0.1N$ silver nitrate and $0.1N$ ammonium thiocyanate.

Exercise 27

Object. To Prepare and Standardize 0.1*N* Silver Nitrate.

Material Required. 10 Gm. of silver nitrate.

Procedure. Dry about 10 Gm. of pure silver nitrate in an electric oven at 110° to constant weight. Weigh out exactly 8.503 Gm. of the dried salt and dissolve it in sufficient distilled water to make 500 cc. at 25°.

The pure analyzed silver nitrate now available on the market makes it possible to prepare the solution directly from the dried salt. Every precaution should be exercised in weighing the silver nitrate, since a slight error in the weight would make the concentration of the solution inaccurate.

Since the univalent silver cation, Ag^+, is, in a sense, a chemical equivalent of the hydrogen ion, 1 mole of $AgNO_3$ (169.89 Gm.) is 1 equivalent. A liter of 0.1*N* silver nitrate should, therefore, contain 16.989 Gm. of $AgNO_3$ and 500 cc., 8.4945 Gm. If the solution is prepared as directed at 25° in glassware which has been calibrated at 20°, 8.503 Gm. of silver nitrate is used instead of the calculated 8.4945 Gm. to allow for the slight expansion of the glass vessel at the higher temperature.

If the purity of the silver nitrate crystals used is doubtful, the concentration of the volumetric solution may be determined by titration against an accurately weighed sample of pure sodium chloride in the same manner as given under the assay of sodium chloride.

The distilled water should be tested for halides as follows, before it is used to prepare standard silver nitrate solution:

Fill a test tube three-fourths full of water, add about 0.5 cc. of silver nitrate solution and a few drops of nitric acid. Any turbidity is positive evidence that the water contains halides and is unfit for use in preparing the standard solution, unless the concentration of the solution is to be determined by titration against a pure chloride.

Exercise 28

Object. To Prepare and Standardize 0.1*N* Ammonium Thiocyanate.

Materials Required. 4.0 Gm. of reagent ammonium thiocyanate. 0.1*N* silver nitrate.

2 cc. of ferric ammonium sulfate T.S.

Procedure. Dissolve about 4 Gm. of reagent ammonium thiocyanate in 500 cc. of water. Place 30.00 cc. of 0.1*N* silver nitrate in a 250-cc. Erlenmeyer flask. Dilute with 50 cc. of water, then add 2 cc. of nitric acid and 2 cc. of ferric ammonium sulfate T.S. and titrate with the ammonium thiocyanate solution to the first appearance of a red brown color. Calculate the normality of the ammonium thiocyanate solution, and, if desired, adjust the solution to exactly 0.1*N*.

Since ammonium thiocyanate is deliquescent, a slight amount in excess of the theoretical quantity required is used in preparation of the solution. As the sample of silver nitrate solution is titrated with the ammonium thiocyanate solution, a white precipitate of AgSCN is formed. As the end point is approached, the precipitate becomes flocculent and settles readily. At the end point, a drop of ammonium thiocyanate solution produces a permanent reddish-brown color which does not disappear on shaking. Equations for the reactions involved follow.

$$AgNO_3 + NH_4SCN \longrightarrow \underset{\text{white}}{AgSCN} + NH_4NO_3$$

$$FeNH_4(SO_4)_2 + 3NH_4SCN \longrightarrow Fe(SCN)_3 + 2(NH_4)_2SO_4$$

Oxides of nitrogen give colored salts with ferric alum. Solutions containing oxides of nitrogen and also the nitric acid employed as above should be boiled prior to the addition of the indicator.

Questions and Problems

1. Explain in detail how to standardize a solution of silver nitrate against pure sodium chloride.

2. Why is nitric acid added when NH_4SCN solution is standardized against 0.1*N* $AgNO_3$ using ferric alum as indicator?

3. Write ionically the reactions involved in the standardization of NH_4SCN against $AgNO_3$ with ferric alum indicator.

4. What must be the normality of a solution of silver nitrate so that each cubic centimeter shall be equivalent to 6 mg. of NaCl?

5. How might 0.1*N* $AgNO_3$ be prepared from pure metallic silver?

6. Why should standard silver nitrate solution be protected from strong sunlight?

7. Why must oxides of nitrogen be absent in titrations with NH_4SCN?

8. How much 0.1*N* $AgNO_3$ could be prepared from 5 Gm. of pure silver?

9. Calculate the weight (milligrams) of each of the following that is equivalent to 1 cc. of 0.1*N* ammonium thiocyanate: (*a*) yellow mercuric oxide; (*b*) silver itrate: (c) mercurial ointment (Hg); (*d*) mild silver protein (Ag). Compare alues with those given in the official monographs.

DIRECT TITRATION METHOD

Compounds of silver and mercury that can be readily converted nto soluble silver or mercury (Hg^{++}) salts, respectively, may be esti-nated by direct titration with standard ammonium thiocyanate olution, using ferric ammonium sulfate as indicator. The method s based on the quantitative precipitation of the corresponding thio-yanate, *e.g.*,

$$AgNO_3 + NH_4SCN \longrightarrow AgSCN + NH_4NO_3$$

nd

$$Hg(NO_3)_2 + 2NH_4SCN \longrightarrow Hg(SCN)_2 + 2NH_4NO_3$$

When all the metallic ion has been precipitated as thiocyanate, he NH_4SCN reacts with the ferric alum indicator to form red-olored ferric thiocyanate, marking the end point of titration:

$$FeNH_4(SO_4)_2 + 3NH_4SCN \longrightarrow Fe(SCN)_3 + 2(NH_4)_2SO_4$$

The solution must be acidified with nitric acid to prevent the ydrolysis that ferric salts undergo in neutral solution. Chlorides nust be absent, since the chlorides of silver and mercury are more oluble than the respective thiocyanates. This method of assay is xemplified in the following exercise.

Exercise 29

Object. Assay of Strong Silver Protein.

Materials Required. 2 Gm. of strong silver protein.
About 10 cc. of nitric acid.
2 cc. of ferric alum indicator (8 Gm. of $FeNH_4(SO_4)_2.12H_2O$ in 100 cc.).
0.1*N* ammonium thiocyanate.

Procedure. 1. "Ignite about 2 Gm. of Strong Silver Protein, accurately eighed, in a porcelain crucible until all of the carbon is burned off. Transfer a beaker as much of the residue as possible, add to the crucible 5 cc. of nitric id, warm to dissolve any adhering silver, and transfer the solution to the eaker with the aid of a little water. Cover the beaker, and heat on a water ath until all of the metallic silver is dissolved, adding a little more nitric acid, necessary."

Upon ignition, strong silver protein yields a residue of metallic silver and silver oxide. The silver and silver oxide are dissolved in nitric acid:

$$Ag + 2HNO_3 \longrightarrow AgNO_3 + H_2O + NO_2$$
$$Ag_2O + 2HNO_3 \longrightarrow 2AgNO_3 + H_2O$$

2. "Filter into an Erlenmeyer flask, wash the insoluble residue thoroughly with water, cool, and dilute with water, if necessary, to about 75 cc. Add 2 cc. of ferric ammonium sulfate T.S., and titrate with 0.1*N* ammonium thiocyanate. Each cubic centimeter of 0.1*N* ammonium thiocyanate is equivalent to 10.79 mg. of Ag."

The mixture is filtered to free it of carbonaceous matter, the residue on the filter is washed free of silver nitrate, and the nitrate is titrated with 0.1*N* NH_4SCN:

$$AgNO_3 + NH_4SCN \longrightarrow AgSCN + NH_4NO_3$$

The equivalent weight of silver is 107.88 Gm. since 1 gram-atomic weight of silver produces 1 mole of silver nitrate on ignition of the sample and solution in nitric acid. Each cubic centimeter of 0.1*N* ammonium thiocyanate, 0.1 meq., is equivalent to $0.1 \times 0.10788 = 0.010788$ Gm. or 0.1 meq. of Ag. The N.F. specifies that strong silver protein contain 7.5 to 8.5 per cent of Ag. Calculate the percentage of silver in the sample analyzed and compare the result with the N.F. requirement.

Questions and Problems

1. Calculate the following titer values for 0.1*N* ammonium thiocyanate and compare with the values given in the official monographs: (*a*) yellow mercuric oxide, HgO; (*b*) meralluride, $C_9H_{16}HgN_2O_2$; (*c*) mild mercurous chloride ointment, HgCl; (*d*) thimerosal ointment, $C_9H_9HgNaO_2S$.

2. What preliminary treatment of samples of each of the following is necessary in order to obtain a solution of an equivalent amount of silver or mercury salt prior to titration? (*a*) Mersalyl; (*b*) mercury mass; (*c*) phenylmercuric chloride. Explain, by equation if possible, the purpose of the reagents employed.

3. In titration of a solution of the ash from 2.4090-Gm. sample of strong silver protein in nitric acid, 18.14 cc. of a solution of ammonium thiocyanate was required. Each cubic centimeter of the ammonium thiocyanate solution was equivalent to 9.8410 mg. of pure silver. Calculate the Ag content of the sample (per cent).

ABLE 19. OFFICIAL SUBSTANCES ASSAYED BY DIRECT TITRATION WITH 0.1*N* AMMONIUM THIOCYANATE

Substance	Equivalent of 1 cc., mg.	Official requirement, per cent
.S.P.		
Meralluride	22.44	$C_9H_{16}HgN_2O_6$ = 94 to 106*
Meralluride injection	22.44	$C_9H_{16}HgN_2O_6$ = 94 to 106*
Mercuric oxide, yellow	10.83	HgO = (n.l.t.) 99.5
Mercuric oxide, yellow, ointment	10.83	HgO = 0.9 to 1.1
Mercurophylline	22.50	$C_{14}H_{24}HgNNaO_5$ = 94 to 106*
Mercurophylline injection	25.50	$C_{14}H_{24}HgNNaO_5$ = 94 to 106*
Mercurophylline tablets	25.50	$C_{14}H_{24}HgNNaO_5$ = 90 to 110*
Mercury, ammoniated	12.60	$HgNH_2Cl$ = 98 to 100.5
Mersalyl	25.29	$C_{13}H_{16}HgNNaO_6$ = 97 to 103
Mersalyl and theophylline injection	25.29	$C_{13}H_{16}HgNNaO_6$ = 94 to 106*
Mersalyl and theophylline tablets	25.29	$C_{13}H_{16}HgNNaO_6$ = 90 to 110*
Silver nitrate	16.99	$AgNO_3$ = (n.l.t.) 99.8
Silver nitrate ophthalmic solution	3.398†	$AgNO_3$ = 0.95 to 1.05*W/V*
.F.		
Mercurial ointment, mild	10.03	Hg = 9 to 11
Mercurial ointment, strong	10.03	Hg = 47.5 to 52.5
Mercurous chloride, mild, ointment	11.80	HgCl = 28.5 to 31.5
Mercuric oxide, red	10.83	HgO = (n.l.t.) 99.5
Mercuric oxide, red, ointment	10.83	HgO = 9.5 to 10.5
Mercuric salicylate	10.03	Hg = 54 to 59.5
Mercuric salicylate injection	10.03	Hg = 52.5 to 61*
Mercuric succinimide	10.03	Hg = 49.5 to 51.0
Mercuric succinimide injection	10.03	Hg = 48 to 52*
Mercury mass	10.03	Hg = 31 to 35
Mercury oleate	10.83	HgO = 24 to 26
Nitromersol	10.03	Hg = 56.0 to 57.4
Nitromersol solution	10.03	Hg = 0.100 to 0.125*W/V*
Nitromersol tincture	10.03	Hg = 0.250 to 0.316*W/V*
Phenylmercuric chloride	10.03	Hg = 63.5 to 64.5
Phenylmercuric nitrate	10.03	Hg = 62.75 to 63.50
Potassium mercuric iodide	10.03	Hg = 25 to 26
Silver nitrate solution, ammoniacal	10.79	Ag = 28.5 to 30.5
Silver nitrate, toughened	16.99	$AgNO_3$ = (n.l.t.) 94.5
Silver protein, mild	10.79	Ag = 19 to 23
Silver protein, strong	10.79	Ag = 7.5 to 8.5
Thimerosal	10.03	Hg = 48.15 to 49.65
Thimerosal jelly	20.24	$C_9H_9HgNaO_2S$ = 0.0925 to 0.1075
Thimerosal ointment	20.24	$C_9H_9HgNaO_2S$ = 0.095 to0.105

* Per cent of labeled amount.

† Equivalent of 1 cc. of 0.02*N* ammonium thiocyanate.

(n.l.t.) = not less than.

4. What volume of $0.1N$ ammonium thiocyanate would be used in titration to a ferric ammonium sulfate end point, a solution containing silver or mercury ions equivalent to (*a*) 0.7500 Gm. of mercury oleate; (*b*) 1.0500 Gm. of ammoniacal silver nitrate solution; (*c*) 0.4500 Gm. of mercuric succinimide?

5. What weight of each of the following would be chosen as sample so that 30.00 cc. of $0.1N$ ammonium thiocyanate would be required in the titration? (*a*) Silver nitrate; (*b*) mercury mass; (*c*) red mercuric oxide; (*d*) mercuric salicylate.

Exercise 30

Object. Assay of Chiniofon.

Materials Required. 0.4 Gm. of Chiniofon.
15 cc. of sodium hydroxide T.S.
30 cc. of potassium permanganate (1 in 15).
20 cc. of diluted sulfuric acid.
20 cc. of sodium bisulfite (1 in 15).
$0.05N$ silver nitrate.

Procedure. 1. "Place about 400 mg. of Chiniofon, accurately weighed, in a 500-cc. Erlenmeyer flask, and add 15 cc. of sodium hydroxide T.S. Warm gently, and when completely dissolved, add 25 cc. of a solution of potassium permanganate (1 in 15). Add several glass beads, place a small short-stemmed funnel in the mouth of the flask, and boil gently for 10 minutes."

Chiniofon is a mixture of 7-iodo-8-hydroxyquinoline-5-sulfonic acid and its sodium salt. In the oxidative degradation of the organic compounds by heating with alkaline permanganate, the organically bound iodine is converted to an equivalent amount of iodate (IO_3^-).

2. "Allow the mixture to cool to room temperature, wash the funnel and walls of the flask with 75 cc. of water, and add 10 cc. of sulfuric acid (1 in 2). Add, in one portion, 15 cc. of a solution of sodium bisulfite (1 in 5), and, when the solution has become colorless, cool, and then add the solution of potassium permanganate, dropwise, until a yellow color appears. At once add the solution of sodium bisulfide, dropwise, until the yellow color is again discharged. Now add, dropwise, a dilute solution of potassium permanganate, prepared by mixing 1 cc. of the 1 in 15 solution with 49 cc. of water, until a faint yellow color appears."

The mixture is acidified and treated with sodium bisulfite to reduce the iodate to iodide; at the same time the excess $KMnO_4$ and the MnO_2 present are reduced to Mn^{++}. The resulting colorless solution is treated alternately with potassium permanganate and sodium bisulfite until a very small amount of free iodine is present as indicated by a faint yellow color. Practically all the iodine from the organic iodine compound must be present in this adjusted solution as iodide.

> 3. "Add 1 cc. of starch T.S., and titrate with 0.05*N* silver nitrate until the blue color is just discharged, leaving a canary yellow precipitate. Each cubic centimeter of 0.05*N* silver nitrate is equivalent to 6.346 mg. of iodine."

When starch is added a blue color appears because both iodine and iodide are present. As silver nitrate solution is run into the mixture, the color gradually fades to green and the precipitated silver iodide coagulates and adsorbs the green color near the end point. At the end point, the precipitate is canary yellow. Such an end point occurs when the silver nitrate has reacted with all the iodide present including that formed by the reaction

$$3I_2 + 3H_2O \rightleftharpoons HIO_3 + 5H^+I^-$$

A small proportion of iodine and iodate is still present at the end point so that it occurs slightly before the stoichiometric point is reached, and results which are slightly low are obtained.

The equivalent weight of iodine when assayed in this way is 126.92 Gm., 1 gram-atomic weight. Hence each cubic centimeter of 0.05*N* silver nitrate is equivalent to $0.05 \times 0.12692 = 0.006346$ Gm. of iodine.

In Table 20 are listed a number of organic iodine compounds which are assayed by methods like that used for Chiniofon. The use of dichlorofluorescein in direct titrations with standard silver nitrate is exemplified in the following exercise.

Exercise 31

Object. Assay of Sodium Iodide Injection.

Materials Required. 1 ampul of sodium iodide injection.
Dichlorofluorescein T.S.
0.1*N* silver nitrate.

Procedure. 1. "Transfer to a suitable container an accurately measured volume of Sodium Iodide Injection, containing about 250 mg. of sodium iodide; add 200 cc. of water and 10 drops of dichlorofluorescein T.S. as the indicator."

If the ampul is labeled 1 Gm. of sodium iodide in 10 cc., a 2.5-cc.

sample, containing 250 mg. of sodium iodide, would be employed in the assay.

2. "Titrate the mixture with 0.1*N* silver nitrate until the silver iodide flocculates and the mixture turns to a faint pink. Carry out the titration in diffused light, preferably from a northern sky, or artificial light, and against a white background. Each cubic centimeter of 0.1*N* silver nitrate is equivalent to 14.99 mg. of NaI."

The silver iodide precipitates as a colloid stabilized by adsorption of iodide ions. As the iodide ion concentration decreases when the end point is approached, the colloid is discharged and flocculation occurs. At the end point, the particles of suspended silver iodide are positively charged because of adsorption of the excess silver ions.

TABLE 20. OFFICIAL ASSAYS BY DIRECT TITRATION WITH STANDARD SILVER NITRATE

Substance	Normality of standard solution	Equivalent of 1 cc., mg.	Official requirement, per cent
U.S.P.			
Chiniofon	0.05	6.346	I = 26.5 to 29.0
Chiniofon tablets	0.05	6.346	I = 25 to 29*
Diiodohydroxyquinoline	0.05	6.346	I = 60.5 to 64.0
Diiodohydroxyquinoline tablets	0.05	9.924	$C_9H_5I_2NO$ = 93 to 107*
Iodized oil	0.1	12.69	I (organically combined) = 38 to 42
Iodoalphionic acid	0.05	12.35	$C_{15}H_{12}I_2O_3$ = 98 to 102
Iodophthalein sodium	0.05	6.346	I = 60 to 63*
Iodopyracet injection	0.05	6.346	I = 61.5 to 63.5*
Methiodal sodium	0.05	12.20	CH_2INaO_3S = 98 to 102
Methiodal sodium injection	0.05	12.20	CH_2INaO_3S = 95 to 105*
Sodium iodomethamate	0.05	6.346	I = 50.5 to 52.5
Sodium iodomethamate injection	0.05	12.31	$C_8H_3I_2NNa_2O_5$ = 95 to 105*
N.F.			
Calcium iodobehenate	0.1	12.69	I = (n.l.t.) 23.5
Iodine ointment	0.1	12.69	I = 6.5 to 7.5
Sodium iodide injection	0.1	14.99	NaI = 95 to 103*
Sodium salicylate and iodide injection	0.1	14.99	NaI = 93 to 103*
Sodium salicylate and iodide with colchicine injection	0.1	14.99	NaI = 93 to 103*

* Per cent of labeled amount.

(n.l.t.) = not less than.

secondary adsorption of the dichlorofluorescein anions results in he development of a pink color. Protection from direct sunlight is ecessary because of the photocatalyzed reduction of silver halides, he resulting increase in iodide ion concentration, and consumption f more silver nitrate solution. Furthermore, the color imparted to he mixture by the colloidally dispersed free silver would confuse the nd-point observation.

The equivalent weight of sodium iodide is 149.92 Gm., 1 mole; herefore, each cubic centimeter of 0.1N silver nitrate, 0.1 meq., is quivalent to 0.1 × 0.14992 = 0.014992 Gm. or 0.1 meq. of NaI.

RESIDUAL TITRATION METHOD (VOLHARD'S METHOD)

This method is based on the complete precipitation of insoluble ilver salts from nitric acid solution by the addition of excess stand- rd silver nitrate solution to a soluble salt and the determination of he amount of silver nitrate solution in excess by residual titration vith standard ammonium thiocyanate solution, using ferric ammo- ium sulfate as indicator.

Exercise 32

To Determine the Purity of a Soluble Chloride. The purity f any soluble chloride may be determined by the method given elow, provided that other substances which give precipitates with ilver nitrate are absent. Substances other than chlorides that pro- luce slightly soluble silver salts are bromides, iodides, cyanides, hiocyanates, sulfides, phosphates, arsenates, carbonates, etc.

Object. Assay of Sodium Chloride.

Materials Required. 1 Gm. of sodium chloride.
3 cc. of nitric acid.
2 cc. of ferric ammonium sulfate T.S.
50 cc. of 0.1N silver nitrate.
0.1N ammonium thiocyanate.
3 cc. of nitrobenzene.

Procedure. "Weigh accurately about 250 mg. of Sodium Chloride, pre- iously dried at 105° for 2 hours, dissolve it in 50 cc. of water in a glass-stop- ered flask, and add 50 cc. of 0.1N silver nitrate, 3 cc. of nitric acid, and 3 cc. f nitrobenzene. Shake well, add 2 cc. of ferric ammonium sulfate T.S., and itrate the excess silver nitrate with 0.1N ammonium thiocyanate. Each cubic entimeter of 0.1N silver nitrate is equivalent to 5.845 mg. of NaCl."

Nitric acid is added to prevent the precipitation of silver as carbonate, phosphate, etc., and to prevent hydrolysis of the ferric alum.

Silver chloride is sufficiently soluble that the following reaction with ammonium thiocyanate can occur during the back titration:

$$AgCl + NH_4SCN \rightleftharpoons AgSCN + NH_4Cl$$

As a result, the ammonium thiocyanate would be consumed not only by the excess silver nitrate present but also, to an appreciable extent, by the silver chloride unless it is removed by filtration prior to the back titration. As a convenient alternative to filtration, nitrobenzene is added to form a film over the precipitated silver chloride particles, thus preventing reaction with the ammonium thiocyanate. Silver bromide and silver iodide are considerably less soluble, and the precipitate does not interfere in the Volhard analysis of soluble bromides and iodides.

The equivalent weight of sodium chloride is 58.45 Gm. since it reacts mole for mole with silver nitrate.

$$\underset{58.45}{NaCl} + AgNO_3 \longrightarrow AgCl + NaNO_3$$

The difference between the total volume of standard silver nitrate employed and the volume consumed by the standard ammonium thiocyanate in the back titration is a measure of the NaCl present. Each cubic centimeter of 0.1*N* silver nitrate, 0.1 meq., which is consumed by sodium chloride is equivalent to $0.1 \times 0.05845 = 0.005845$ Gm. of NaCl.

Questions and Problems

1. Give equations for the reactions which occur in the official methods for analysis of sodium iodide injection (NaI) and dextrose and sodium chloride injection (NaCl). Compare the methods and explain how the end point is determined in each case.

2. State the important difference in the assay procedures for sodium bromide and for sodium chloride. Give an explanation for the difference.

3. Using appropriate solubility product constants, calculate the relative solubilities of AgCl, AgBr, and AgSCN in water.

4. A 0.2182-Gm. sample of sodium chloride was assayed by the Volhard method using 50.00 cc. of 0.0985*N* silver nitrate and 11.75 cc. of 0.1340*N* ammonium thiocyanate. Calculate the per cent NaCl in the sample.

Exercise 33

Object. Assay of Ammonium Bromide.

Materials Required. 0.1 Gm. of ammonium bromide.
2 cc. of nitric acid.
2 cc. of ferric ammonium sulfate T.S.
50 cc. of 0.1*N* silver nitrate.
0.1*N* ammonium thiocyanate.

Procedure. "Dry about 100 mg. of Ammonium Bromide at 105° for 2 hours and weigh accurately. Dissolve it in about 50 cc. of water, add 50 cc. of 0.1*N* silver nitrate, 2 cc. of ferric ammonium sulfate T.S., and 2 cc. of nitric acid. Titrate the excess of silver nitrate with 0.1*N* ammonium thiocyanate. Each cubic centimeter of 0.1*N* silver nitrate is equivalent to 9.796 mg. of NH_4Br."

The above assay is similar to that for chlorides, except that nitrobenzene need not be added since silver bromide is less soluble than silver thiocyanate.

The reactions and calculations are similar to those of the preceding assay. Write equations for all reactions involved and calculate the percentage purity of the ammonium bromide.

Questions and Problems

1. If the K_{sp} of AgBr is 5×10^{-13}, what is the solubility of silver bromide in grams per liter in water and in 10^{-3} *M* silver nitrate? Explain the effect of excess silver nitrate on the completeness of the precipitation of AgBr in the assay of ammonium bromide.

2. If ammonium iodide, 95.6 per cent NH_4I, is to be assayed by a Volhard procedure using 50.00 cc. of 0.0942*N* silver nitrate, what weight of sample should be employed so that 20.00 cc. of 0.1025*N* ammonium thiocyanate would be required in the back titration?

3. A 250-mg. sample of ammonium bromide was found to be equivalent to 25.00 cc. of 0.1*N* silver nitrate in a Volhard determination. What volume of 0.0962*N* sulfuric acid would be required to neutralize the ammonia produced from a 0.4689-Gm. sample of the same material?

Exercise 34

Object. Assay of Hydriodic Acid Syrup.

Materials Required. 25 cc. of hydriodic acid syrup.
40 cc. of 0.1*N* silver nitrate.
5 cc. of nitric acid.
2 cc. of ferric ammonium sulfate T.S.
0.1*N* ammonium thiocyanate.

Procedure. "Place exactly 25 cc. of Hydriodic Acid Syrup in a flask, dilute it with 100 cc. of water, add 40 cc. of $0.1N$ silver nitrate, agitate the mixture, add 5 cc. of nitric acid, and heat on a water bath until the precipitate has acquired a bright yellow color. Cool, add 2 cc. of ferric ammonium sulfate T.S., and determine the residual silver nitrate by titration with $0.1N$ ammonium thiocyanate. Each cubic centimeter of $0.1N$ silver nitrate is equivalent to 12.79 mg. of HI."

This assay is similar to that for bromides except that a measured volume of liquid is used for the sample.

$$\underset{127.93}{HI} + AgNO_3 \longrightarrow AgI + HNO_3$$

$$AgNO_3 + NH_4SCN \longrightarrow AgSCN + NH_4NO_3$$

Calculate the amount of HI equivalent to 1 cc. of $0.1N$ $AgNO_3$ and the per cent of HI in the sample analyzed. The U.S.P. requires that hydriodic acid syrup contain not less than 1.3 and not more than 1.5 Gm. of HI in each 100 cc. Note that this is a weight-to-volume percentage.

Questions and Problems

1. What preliminary treatment is necessary before standard silver nitrate may be added in the Volhard determination of each of the following: (*a*) potassium nitrate, KNO_3; (*b*) tribromoethanol, $C_2H_3Br_3O$; (*c*) sodium nitrite tablets, $NaNO_2$; (*d*) potassium chlorate, $KClO_3$; (*e*) iodoform, CHI_3? Give equations where possible.

2. Calculate the titer values for $0.1N$ silver nitrate for each of the substances indicated in question 1.

3. A silver nitrate solution had a NaCl titer of 5.569 mg. per cc. Each cubic centimeter of the silver nitrate solution would be equivalent to how many milligrams of: (*a*) potassium bromide; (*b*) methacholine chloride; (*c*) strontium bromide; (*d*) sodium thiocyanate; (*e*) potassium chlorate?

4. If 25.00 cc. of hydriodic acid syrup (1.4 per cent W/V HI) were treated with 40.00 cc. of $0.1012N$ silver nitrate, what volume of $0.0968N$ ammonium thiocyanate would be required in the back titration?

5. A 2.00-cc. sample of tribromoethanol solution was assayed by the official method. After adding 50.00 cc. of $0.1N$ silver nitrate, 32.39 cc. of an ammonium thiocyanate solution was required for titration to a ferric alum end point. In a separate titration, 25.00 cc. of the $0.1N$ silver nitrate solution required 23.79 cc. of the ammonium thiocyanate solution. Calculate the per cent W/V of $C_2H_3Br_3O$ in the tribromoethanol solution.

TABLE 21. OFFICIAL SUBSTANCES ASSAYED BY RESIDUAL TITRATION OF 0.1N $AgNO_3$ WITH 0.1N NH_4SCN

Substance	Equivalent of 1 cc. 0.1N $AgNO_3$	Official requirement, per cent
U.S.P.		
Ammonium chloride	5.350	NH_4Cl = (n.l.t.) 99.5
Dextrose and sodium chloride injection	5.845	NaCl = 95 to 105*
Hydriodic acid, diluted	12.79	HI = 9.5 to 10.5W/V
Hydriodic acid syrup	12.79	HI = 1.3 to 1.5W/V
Methacholine chloride	3.546	Cl = 17.8 to 18.4
Methacholine chloride capsules	19.57	$C_8H_{18}ClNO_2$ = 90 to 110*
Methacholine chloride injection	19.57	$C_8H_{18}ClNO_2$ = 90 to 110*
Methamphetamine hydrochloride	3.546	Cl = 18.8 to 19.3
Potassium bromide	11.90	KBr = (n.l.t.) 99
Potassium chloride	7.455	KCl = (n.l.t.) 99
Potassium chloride tablets	7.455	KCl = 95 to 105*
Potassium nitrate	10.11	KNO_3 = (n.l.t.) 99
Ringer's solution		NaCl = 0.82 to 0.90W/V
Ringer's solution, lactated		NaCl = 0.57 to 0.63W/V
Sodium bromide	10.29	NaBr = (n.l.t.) 99
Sodium chloride	5.845	NaCl = (n.l.t.) 99.5
Sodium chloride solution, isotonic	5.845	NaCl = 0.85 to 0.95W/V
Sodium citrate solution, anticoagulant	5.845	NaCl = 0.85 to 0.95W/V
Sodium nitrite tablets	20.70	$NaNO_2$ = 93 to 107*
Tribromoethanol	9.426	$C_2H_3Br_3O$ = (n.l.t.) 99
Tribromoethanol solution	9.426	$C_2H_3Br_3O$ = 99 to 101W/V
N.F.		
Ammonium bromide	9.796	NH_4Br = (n.l.t.) 99
Ammonium bromide elixir	9.796	NH_4Br = 8 to 9W/V
Ammonium chloride tablets	5.350	NH_4Cl = 92.5 to 107.5*
Ammonium iodide	14.50	NH_4I = (n.l.t.) 98
Bromides, five, elixir	7.992	Br = 18.5 to 20W/V
Bromides, three, elixir	10.59	Bromides (total) = 23 to 25W/V
Bromides, three, tablets	7.992	Br = 70 to 81*
Dibucaine hydrochloride	3.647	HCl = 9.5 to 9.7
Ferric chloride solution	3.647	HCl = 3 to 5
Ferrous iodide syrup	15.48	FeI_2 = 6.5 to 7.5W/V
Iodoform	13.13	CHI_3 = (n.l.t.) 99
Lithium bromide	8.686	LiBr = (n.l.t.) 99
Potassium bromide elixir	11.90	KBr = 16.5 to 18.5
Potassium chlorate	12.26	$KClO_3$ = (n.l.t.) 99
Potassium chlorate tablets	12.26	$KClO_3$ = 94 to 106*
Potassium thiocyanate	9.717	KSCN = (n.l.t.) 99
Sodium bromide elixir	10.29	NaBr = 16.5 to 18.5W/V
Sodium bromide tablets	10.29	NaBr = 92.5 to 107.5*
Sodium chloride and dextrose tablets	5.845	NaCl = 92.5 to 107.5*
Sodium chloride tablets	5.845	NaCl = 92.5 to 105*
Sodium thiocyanate	8.109	NaSCN = (n.l.t.) 98.5
Strontium bromide	17.78	$SrBr_2.6H_2O$ = (n.l.t.) 98

* Per cent of labeled amount.

(n.l.t.) = not less than.

Chapter 8

OXIDATION-REDUCTION METHODS

The chemical reactions that occur in neutralization and precipitation methods of analysis take place without change in valence. Oxidation-reduction methods of analysis nearly always involve a change in valence of the reacting substances. A study of the principles underlying the latter type of reaction is an essential preliminary if the methods based on them are to be understood.

Theory. The simplest type of oxidation-reduction reaction is the direct combination of elements. Thus, when oxygen gas unites with hydrogen gas, forming water, $O_2 + 2H_2 \longrightarrow 2H_2O$, the oxygen is reduced and the hydrogen oxidized. When carbon burns in the presence of oxygen to form carbon dioxide, $C + O_2 \longrightarrow CO_2$, it is oxidized and the oxygen is reduced, and when carbon unites with sulfur to form carbon disulfide, $C + 2S \longrightarrow CS_2$, the carbon is oxidized and the sulfur reduced.

From the above simple illustrations, it is obvious that reduction need not imply a reaction of hydrogen, since oxygen is reduced by carbon, and that oxidation need not imply a reaction of oxygen, since carbon is oxidized by sulfur. When one substance is oxidized, some other substance must be correspondingly reduced, and, conversely, when one substance is reduced, some other substance must be correspondingly oxidized.

The oxidation-reduction reactions that take place in the official assay processes are, for the most part, between electrolytes in aqueous solution. Their quantitative value is based upon the fact that metals, nonmetals, and their ions, under suitable conditions, can be made to undergo a change in the quantity of electric charge associated with them and that in the change there exists a simple relationship between the quantity of electricity lost or gained and the weight of the reacting substances. This relationship may be expressed according to Faraday's law as follows: *A change of charge of one is equivalent to the gain or loss of 96,500 coulombs of electricity for each formula weight of element or group of elements involved.* Since in every oxidation-reduction reaction the charge lost or gained by one substance must necessarily be gained or lost by another, it follows that there is always a transfer of electrons in oxidation-reduction reactions.

The reactant which loses electrons in an oxidation-reduction reaction is the *reducing agent* and can be identified in the equation for the reaction as the reactant containing a constituent atom or atoms converted to a higher state of oxidation, *e.g.*,

$$\mathrm{Fe^{++}} \longrightarrow \mathrm{Fe^{3+}} + e$$
$$2\mathrm{I^-} \longrightarrow \mathrm{I_2} + 2e$$

The electrons lost by the reducing agent are gained by the *oxidizing agent*, the reactant containing a constituent atom or atoms which are converted to a lower state of oxidation, *e.g.*,

$$\mathrm{Ce^{4+}} + e \longrightarrow \mathrm{Ce^{3+}}$$
$$\mathrm{Fe^{3+}} + e \longrightarrow \mathrm{Fe^{++}}$$

A combination of oxidizing and reducing agents results in a reaction which may be used as basis for the quantitative measurement of one of the reactants. For example, ferrous sulfate may be assayed by its reaction with ceric sulfate.

$$\mathrm{Fe^{++}} + \mathrm{Ce^{4+}} \longrightarrow \mathrm{Fe^{3+}} + \mathrm{Ce^{3+}}$$

This equation may be divided into two half equations showing the loss of electrons by the ferrous ion and the gain of electrons by the ceric ion

$$\mathrm{Fe^{++}} \rightleftharpoons \mathrm{Fe^{3+}} + e \qquad \text{and} \qquad \mathrm{Ce^{4+}} + e \rightleftharpoons \mathrm{Ce^{3+}}$$

Ferric chloride may be assayed by its reduction with potassium iodide:

$$2\mathrm{I^-} \longrightarrow \mathrm{I_2} + 2e \qquad \text{and} \qquad 2\mathrm{Fe^{3+}} + 2e \longrightarrow 2\mathrm{Fe^{++}}$$

The combined equation may be represented

$$2\mathrm{I^-} + 2\mathrm{Fe^{3+}} \longrightarrow \mathrm{I_2} + 2\mathrm{Fe^{++}}$$

The amount of the reducing agent or oxidizing agent in the sample can be determined by measuring a chemically equivalent amount of the oxidizing agent or reducing agent, respectively.

In the equation for the oxidation of ferrous sulfate with ceric sulfate, each molecule of ferrous sulfate, on oxidation, loses one electron. One mole of ferrous sulfate, therefore, loses 6.02×10^{23} electrons equivalent to 1 faraday or 96,500 coulombs. This quantity of electricity is identical with that necessary to reduce 1 mole of ceric sulfate. Consequently 1 mole of ferrous sulfate and 1 mole of ceric sulfate are chemical equivalents. One gram of hydrogen, acting as a reducing agent, loses electrons equivalent to 96,500 coulombs. Therefore, the *equivalent weight of a reducing agent* is that weight which loses electrons equivalent to 96,500 coulombs, and it can be calculated by dividing the gram-molecular weight by the number of electrons lost by each molecule; *e.g.*, the equivalent weight of ferrous sulfate oxidized to ferric sulfate, $Fe^{++} \longrightarrow Fe^{3+} + e$, is 151.92 Gm. or 1 gram-molecular weight.

An *equivalent weight of an oxidizing agent* is that weight which gains electrons equivalent to 1 faraday, or to the electrons gained by 1 Gm. of hydrogen ions, $2H^+ + 2e \longrightarrow H_2$. It can be calculated by dividing the gram-molecular weight by the number of electrons gained by each molecule; *e.g.*, the equivalent weight of ceric sulfate, $Ce^{4+} + e \longrightarrow Ce^{3+}$, is 1 gram-molecular weight, 332.26 Gm., and that of potassium permanganate, $MnO_4^- + 5e \longrightarrow Mn^{++}$, is $\frac{1}{5}$ gram-molecular weight or 31.61 Gm.

Standard Solutions. The standard solutions used in the official assays by oxidation-reduction methods include: potassium permanganate, potassium dichromate, potassium bromate, potassium iodate, potassium ferricyanide, ceric sulfate, iodine and bromine as oxidizing agents, and oxalic acid, potassium arsenite, and sodium thiosulfate as reducing agents.

The number of electrons gained by a given oxidizing agent sometimes depends on the conditions, *e.g.*, pH, under which the reaction is carried out. Certain oxidizing agents, nitric acid, for example, do not undergo a single uniform reaction even though conditions are carefully controlled. Such substances cannot be used as standard oxidizing agents or assayed by oxidation-reduction methods. The equivalent weights of oxidizing agents which determine the concentrations of standard solutions can be calculated as indicated in the equations

1. Permanganate ion⟶manganous ion
   $MnO_4^- + 5e \longrightarrow Mn^{2+}$

Since each molecule of $KMnO_4$ gains 5 electrons when permanganate is reduced in acid mediums, $\frac{1}{5}$ mole, 158.03/5 = 31.61 Gm., is 1 equivalent. A liter of 0.1*N* solution should contain, therefore, 3.161 Gm. of $KMnO_4$.

2. Dichromate ion⟶chromic ion
   $Cr_2O_7^{=} + 6e \longrightarrow 2Cr^{3+}$

One-sixth mole, 294.21/6 = 49.035 Gm., is 1 equivalent of potassium dichromate, $K_2Cr_2O_7$.

3. Bromate ion⟶bromide ion
   $BrO_3^- + 6e \longrightarrow Br^-$ (in presence of H_2SO_4)

One-sixth mole, 167.01/6 = 27.84 Gm., is 1 equivalent of potassium bromate, $KBrO_3$.

4. Ceric ion⟶cerous ion
   $Ce^{4+} + e \longrightarrow Ce^{3+}$

One mole, 332.25 Gm., is 1 equivalent of ceric sulfate, $Ce(SO_4)_2$.

5. Iodine⟶iodide ion
   $I_2 + 2e \longrightarrow 2I^-$

One-half mole or 1 gram-atomic weight, 126.92 Gm., is 1 equivalent of iodine.

The reduction of the iodine in potassium iodate to lower oxidation states varies considerably depending on conditions specified for the various assays in which it is employed. Therefore, the concentrations of standard solutions of potassium iodate are usually expressed in terms of molarity rather than normality.

Reducing agents used for the preparation of standard solutions are oxidized:

1. Oxalate ion⟶carbon dioxide
   $C_2O_4^{=} \longrightarrow 2CO_2 + 2e$

One-half mole, 126.07/2 = 63.035 Gm., is 1 equivalent of oxalic acid, $H_2C_2O_4.2H_2O$.

2. Thiosulfate ion⟶tetrathionate ion

$$2S_2O_3^{=} \longrightarrow S_4O_6^{=} + 2e$$

The net oxidation number (valence number) of the four sulfur atoms in two thiosulfate ions is 8^+, while that of the four sulfur atoms in the tetrathionate is 10^+; hence two thiosulfate ions must lose 2 electrons in forming one tetrathionate ion, or each thiosulfate ion loses 1 electron. One mole, 248.19 Gm., is 1 equivalent of sodium thiosulfate, $Na_2S_2O_3.5H_2O$.

Permanganate Methods. Potassium permanganate solution can be standardized easily, and it retains its concentration over long periods of time when proper precautions are observed in its preparation and preservation. The reactions of permanganate in solution are rapid. It also serves as an indicator in titrations where it is used, since a very slight excess of permanganate imparts to solutions a distinct pink color.

Exercise 35

Object. To Prepare and Standardize 0.1*N* Potassium Permanganate.

Materials Required. About 3.3 Gm. of potassium permanganate.
About 1 Gm. of reagent sodium oxalate.

Procedure. 1. "Dissolve about 3.3 Gm. of potassium permanganate in 1000 cc. of water in a flask and boil the solution for about 15 minutes. Stopper the flask and allow it to stand for at least 2 days before filtering through asbestos."

Any organic matter that may be present in distilled water is decomposed by potassium permanganate. Consequently, after solution of the permanganate is effected, the liquid is allowed to stand for 2 days to ensure the completion of the decomposition reaction. The solution is then filtered through asbestos to remove all traces of manganese dioxide, which if present acts as a catalyst to promote the formation of more manganese dioxide at the expense of permanganate ion. The formation of MnO_2 as a reduction product in neutral solution may be represented

$$4KMnO_4 + 2H_2O \longrightarrow 4KOH + 4MnO_2 + 3O_2$$

2. "Ascertain its exact normality by titration against reagent sodium oxalate as follows:

"Weigh accurately about 200 mg. of reagent sodium oxalate, previously dried to constant weight at 110°, and dissolve it in 250 cc. of water. Add 7 cc. of sulfuric acid, heat to about 70° and then slowly add the permanganate solution from a burette, with constant stirring, until a pale pink color is produced which persists for 15 seconds. The temperature at the conclusion of the titration should not be less than 60°. Calculate the normality of the solution and, if desired, adjust the solution exactly to 0.1*N*."

Titrate the mixture by adding the permanganate solution from a glass-stoppered burette, with continuous stirring of the titration mixture until a drop of the permanganate causes a permanent pink color. Read the top of the meniscus in the burette instead of the lowest part. Using the first sample as a preliminary run, titrate two other portions of oxalate in the same manner and base the calculations of normality upon them.

Sodium oxalate is the best standard to use in the standardization of potassium permanganate, since it can be obtained in very pure condition. Sufficient sulfuric acid must be added to keep the hydrogen ion concentration reasonably constant throughout the titration, thereby preventing the formation of manganese dioxide and supplying the hydrogen ions used up in the reduction of the permanganate ion. Thus, for every mole of permanganate ion reduced, 8 moles of hydrogen ion are required, as shown in the equation below. The top of the meniscus is read, since the color of the permanganate solution renders impossible accurate readings of the lowest point.

The half equations are:

$$MnO_4^- + 8H + 5e \longrightarrow Mn^{++} + 4H_2O$$

and

$$C_2O_4^{=} \longrightarrow 2CO_2 + 2e$$

In order to balance and cancel out electrons the half equation for reduction of permanganate is multiplied by 2 and that for the oxidation of oxalate is multiplied by 5. The combined equation may be written $2MnO_4^- + 16H^+ + 5C_2O_4^{=} \longrightarrow 2Mn^{++} + 8H_2O + 10CO_2$ or

$$5Na_2C_2O_4 + 2KMnO_4 + 8H_2SO_4 \longrightarrow 2MnSO_4 + 5Na_2SO_4 + K_2SO_4 + 10CO_2 + 8H_2O$$

The balanced equation shows that 5 molecules of sodium oxalate lose 10 electrons on oxidation with potassium permanganate; therefore, the equivalent weight of sodium oxalate is one-half the gram-molecular weight, or 134.01/2 = 67.01 Gm. If a 0.2500-Gm. sample of sodium oxalate required 34.20 cc. of a permanganate solution in a titration, there must have been 0.2500/67.01 equivalents of $KMnO_4$ in the 34.20 cc. of the solution, or

$$\frac{1{,}000 \times 0.2500}{34.20 \times 67.01} = 0.1091 \text{ equivalent of } KMnO_4 \text{ per liter}$$

Hence the permanganate solution is $0.1091N$.

When permanganate solutions are diluted to an exact normality, water freshly distilled from permanganate solution should be used to avoid the introduction of organic matter.

Potassium permanganate solution prepared as directed will retain its value for a long time if it is protected from sunlight and from the entry of organic matter. It is best, however, to restandardize the solution from time to time. Solution of standard potassium permanganate withdrawn from the stock bottle should never be returned to it. Solutions of oxidizing agents should never be titrated from Mohr burettes, since the oxidizing agent attacks the rubber and the solutions are thereby weakened in concentration. The burette used to measure the permanganate solution should be washed out immediately after use to prevent the formation of a deposit of manganese dioxide on the burette wall.

Questions and Problems

1. Define the following terms: (*a*) oxidation; (*b*) reducing agent; (*c*) equivalent weight of an oxidizing agent.

2. Refer to the U.S.P. and give the formula and equivalent weight of the primary standards used in standardizing (*a*) $0.1N$ iodine; (*b*) $0.1N$ potassium permanganate; (*c*) $0.1N$ ceric sulfate.

3. A 0.2295-Gm. sample of sodium oxalate (97.8 per cent $Na_2C_2O_4$) required 35.12 cc. of a potassium permanganate solution in a titration. What was the normality of the permanganate solution?

4. What volume of $0.1225N$ sodium hydroxide would be required in titration of a 0.1065-Gm. sample of oxalic acid if a 0.1126-Gm. sample of the oxalic acid required 24.65 cc. of $0.0965N$ potassium permanganate in a titration?

DIRECT TITRATION METHODS

Exercise 36

To Determine the Purity of Ferrous Sulfate. Potassium permanganate is especially well suited to the estimation of iron and ferrous salts oxidimetrically. When the titration is carried out in solution acidified with sulfuric acid, the ferrous ion is oxidized quantitatively to ferric ion.

Object. Assay of Ferrous Sulfate.

Materials Required. About 1 Gm. of ferrous sulfate.
25 cc. of diluted sulfuric acid.
$0.1N$ potassium permanganate.

Procedure. "Dissolve about 1 Gm. of Ferrous Sulfate, accurately weighed, in 25 cc. of diluted sulfuric acid and 25 cc. of water, and titrate with $0.1N$ potassium permanganate until a permanent pink color is produced. Each cubic centimeter of $0.1N$ potassium permanganate is equivalent to 15.19 mg. of $FeSO_4$ or to 27.80 mg. of $FeSO_4.7H_2O$."

After dissolving the sample in 25 cc. of diluted sulfuric acid, it is best to wash down the sides and neck of the flask with about 25 cc. of cold water. The titration must be conducted in acid solution to maintain a reasonably constant hydrogen ion concentration, as explained on page 163. The pink color of permanganate is distinct when a drop in excess of that required to oxidize all the ferrous salt to the ferric state has been added.

Balancing the equation for the reactions that occur may be achieved by writing the reactions as two half equations, one showing the gain of electrons by the oxidizing agent and the other showing the loss of electrons by the reducing agent, *e.g.*:

$$5Fe^{++} \longrightarrow 5Fe^{3+} + 5e$$
$$MnO_4^- + 8H^+ + 5e \longrightarrow Mn^{++} + 4H_2O$$

adding the two half equations gives

$$MnO_4^- + 8H^+ + 5Fe^{++} \longrightarrow Mn^{++} + 4H_2O + 5Fe^{3+}$$

If the complete molecular equation is required the combined half equations must be multiplied by 2:

$$2KMnO_4 + 8H_2SO_4 + 10FeSO_4 \longrightarrow 2MnSO_4 + 8H_2O + 5Fe_2(SO_4)_3 + K_2SO_4$$

The equation reveals that 10 molecules of ferrous sulfate lose 10 electrons; hence the equivalent weight of $FeSO_4$ is 1 mole, 151.91 Gm., and that of $FeSO_4.7H_2O$ is 278.03 Gm. Each cubic centimeter of 0.1*N* potassium permanganate, 0.1 meq., is equivalent to $0.1 \times 0.15191 = 0.015191$ Gm. or 0.1 meq. of $FeSO_4$, or to $0.1 \times 0.27803 = 0.027803$ Gm. of $FeSO_4.7H_2O$. If the amount of iron present in the sample in the Fe^{++} state is desired, its equivalent weight would be 55.85 Gm. because 1 gram-atomic weight of iron is equivalent to 1 mole of ferrous sulfate.

Calculate the per cent of $FeSO_4.7H_2O$ in the sample assayed and compare the value with the official specifications.

The reduction of an equivalent amount of permanganate by Fe^{++} is employed in the assay of titanium dioxide for TiO_2. A solution of the titanium dioxide sample in diluted sulfuric acid is passed through a Jones reductor packed with amalgamated zinc and, during the passage, Ti^{4+} is reduced quantitatively to Ti^{3+}. The solution containing an equivalent amount of Ti^{3+} must be collected in an inert atmosphere because of air oxidation of the Ti^{3+} ion. As a more convenient alternative, the N.F. directs that the solution be collected in ferric ammonium sulfate T.S. When this is done, the Ti^{3+} reduces an equivalent amount of Fe^{3+} to Fe^{++}, which is reasonably stable in air and can be measured by titration with standard permanganate.

Questions and Problems

1. What weight of exsiccated ferrous sulfate, 80 per cent $FeSO_4$, would be used as sample so that 35.00 cc. of 0.1*N* potassium permanganate would be required in the titration? Compare the calculated value with the amount specified in the U.S.P. assay procedure.

2. Calculate the per cent of iron present as Fe^{++} in ferrous sulfate, 99.8 per cent $FeSO_4.7H_2O$, and in exsiccated ferrous sulfate, 86 per cent $FeSO_4$.

3. Consult available texts on volumetric analysis and suggest an indicator which may be used when titrating ferrous sulfate with very dilute standard permanganate solutions.

4. A 1.1021-Gm. sample of iron wire was dissolved in hydrochloric acid and the solution treated with zinc in such a way that all the iron was present as Fe^{++}. The resulting solution required 37.63 cc. of 0.5220*N* potassium permanganate in a titration. Calculate the Fe content of the sample.

Exercise 37

Object. Assay of Hydrogen Peroxide Solution.

Materials Required. About 2 cc. of hydrogen peroxide solution.
20 cc. of diluted sulfuric acid.
0.1*N* potassium permanganate.

Procedure. "Measure accurately 2 cc. of Hydrogen Peroxide Solution, and transfer it to a suitable flask containing 20 cc. of water. Add 20 cc. of diluted sulfuric acid, and titrate with 0.1*N* potassium permanganate. Each cubic centimeter of 0.1*N* potassium permanganate is equivalent to 1.701 mg. of H_2O_2."

The reasons for the various steps in the above assay have already been given under the assay of ferrous sulfate.

Although hydrogen peroxide generally acts as an oxidizing agent and is reduced to H_2O, in this assay it reduces the potassium permanganate and is oxidized, yielding O_2.

$$\underset{34.02}{5H_2O_2} + 2KMnO_4 + 3H_2SO_4 \longrightarrow 5O_2 + 2MnSO_4 + K_2SO_4 + 8H_2O$$

The equation shows that 1 molecule of H_2O_2 loses 2 electrons; hence the equivalent weight of H_2O_2 is ½ gram-molecular weight or 17.01 Gm. Consequently each cubic centimeter of 0.1*N* potassium

TABLE 22. OFFICIAL SUBSTANCES ASSAYED BY DIRECT TITRATION WITH 0.1*N* $KMnO_4$

Substance	Equivalent of 1 cc., mg.	Official requirement, per cent
U.S.P.		
Ferrous sulfate	27.60	$FeSO_4.7H_2O$ = 99.5 to 104.5
Ferrous sulfate, exsiccated	15.19	$FeSO_4$ = (n.l.t.) 80
Hydrogen peroxide solution	1.701	H_2O_2 = 2.5 to 3.5*W/V*
N.F.		
Sodium perborate	0.8000	0 (available) = (n.l.t.) 9
Sodium perborate, monohydrated	0.8000	0 (available) = (n.l.t.) 15.5

(n.l.t.) = not less than.

permanganate is equivalent to $0.1 \times 0.01701 = 0.001701$ Gm. or 0.1 meq. of H_2O_2.

To comply with U.S.P. definition, solution of hydrogen peroxide should contain not less than 2.5 and not more than 3.5 per cent *W/V* of H_2O_2. Calculate the percentage of H_2O_2 in the sample assayed and compare the strength of the solution with the U.S.P. requirement.

Questions and Problems

1. A 2.00-cc. sample of hydrogen peroxide solution required 8.5 cc. of a permanganate solution in titration. If each cubic centimeter of the permanganate solution is equivalent to 0.007294 Gm. of Fe, what per cent *W/V* of H_2O_2 was in the sample?

2. When sodium perborate, $NaBO_3.4H_2O$, is assayed by titration with standard permanganate, each mole is equivalent to 1 mole of H_2O_2: $NaBO_3.4H_2O \longrightarrow H_2O_2 + NaBO_2 + 3H_2O$. (*a*) How many grams of "available" oxygen are in 10 Gm. of sodium perborate? (*b*) If a sodium perborate sample were found on analysis to contain 9.0 per cent "available" oxygen, what was the $NaBO_3.4H_2O$ content of the sample?

3. If 31.00 cc. of a potassium permanganate solution were required in titration of 0.4617 Gm. of 85 per cent $NaBO_3.4H_2O$, what volume of the permanganate solution would be required in titration of 2.00 cc. of 3 per cent H_2O_2, *W/V*?

INDIRECT TITRATION METHODS

Exercise 38

Object. Assay of Calcium Gluconate.

Materials Required. 0.5 Gm. of calcium gluconate.
2 cc. of hydrochloric acid.
Ammonium oxalate T.S. (3.5 Gm. ammonium oxalate in 100 cc. of water).
Dilute sulfuric acid (1 in 3).
0.1*N* potassium permanganate.

Procedure. 1. "Dissolve about 500 mg. of Calcium Gluconate, accurately weighed, in 100 cc. of water and 2 cc. of hydrochloric acid, heat almost to boiling, and add 15 cc. of ammonium oxalate T.S. and 2 drops of methyl red T.S. To the hot solution, constantly stirred, add, dropwise, from a burette, dilute ammonia T.S. (1 in 5) until the mixture is just alkaline, and let stand for 2 hours. Decant the clear supernatant liquid through a hardened filter paper, and completely transfer the precipitate to the filter by means of cold water. Wash the precipitate with small portions of cold water until the filtrate shows no turbidity within 1 minute after the addition of calcium chloride T.S."

By treatment of the calcium gluconate sample with ammonium oxalate, an equivalent amount of reasonably pure calcium oxalate, $CaC_2O_4.H_2O$, is precipitated.

$$\underset{448.39}{C_{12}H_{22}CaO_{14}.H_2O} + (NH_4)_2C_2O_4 \longrightarrow CaC_2O_4.H_2O + 2CH_2OH(CHOH)_4CO_2NH_4$$

Care must be exercised during the precipitation to prevent coprecipitation of sodium, potassium, and magnesium oxalates. When the ammonium oxalate is added to the acid solution of calcium gluconate, the $C_2O_4^{--}$ ion concentration is low because of the high basicity of the oxalate ion, $C_2O_4^{--} + H^+ \rightleftharpoons HC_2O_4^-$. As ammonia T.S. is added slowly with stirring to the hot solution, the $CaC_2O_4.H_2O$ crystals form slowly; are not contaminated with coprecipitated sodium, potassium, or magnesium oxalates; and grow sufficiently large so that filtration is readily accomplished. When the color of the methyl red changes from red to yellow, the pH is such that the oxalate ion concentration is sufficiently high for complete precipitation of the calcium ions. The mixture is allowed to stand for 2 hr. to ensure complete precipitation but should not be permitted to stand longer since magnesium oxalate may begin to precipitate also.

2. "Puncture the filter, and transfer the precipitate to a beaker by means of hot water dispensed from a wash bottle equipped with a fine tip. Wash the filter paper thoroughly with 30 cc. of hot dilute sulfuric acid (1 in 3) directed in a fine stream under pressure, and then wash the filter thoroughly with hot water. A glass filtering crucible may be used instead of a paper filter. Heat the combined washings to 80°, and titrate with 0.1*N* potassium permanganate. Each cubic centimeter of 0.1*N* potassium permanganate is equivalent to 22.42 mg. of $C_{12}H_{22}CaO_{14}.H_2O$."

The $CaC_2O_4.H_2O$, equivalent to the calcium gluconate in the sample, could be measured gravimetrically after drying carefully at 100° or after ignition to carbonate at 450 to 550° or to oxide at higher temperatures. However, because of difficulties in obtaining uniform substances in this way, the titrimetric procedure is generally more accurate as well as more convenient.

Calcium oxalate is soluble in sulfuric acid at 80°, the solution reduces permanganate at a sufficiently rapid rate for successful titration.

$$5CaC_2O_4 + 2KMnO_4 + 8H_2SO_4 \longrightarrow 10CO_2 + 2MnSO_4 + 5CaSO_4 + K_2SO_4 + 8H_2O$$

TABLE 23. OFFICIAL SUBSTANCES ASSAYED BY PRECIPITATION WITH AMMONIUM OXALATE AND TITRATION OF THE OXALIC ACID FROM THE PRECIPITATE WITH 0.1*N* $KMnO_4$

Substance	Equivalent of 1 cc., mg.	Official requirement, per cent
U.S.P.		
Calcium carbonate, precipitated	5.005	$CaCO_3$ = (n.l.t.) 98.5
Calcium chloride	7.351	$CaCl_2.2H_2O$ = 99 to 107
Calcium gluconate	22.42	$C_{12}H_{22}CaO_{14}.H_2O$ = (n.l.t.) 99
Calcium gluconate injection	22.42	$C_{12}H_{22}CaO_{14}.H_2O$ = 95 to 105[*]
Calcium gluconate tablets	22.42	$C_{12}H_{22}CaO_{14}.H_2O$ = 95 to 105[*]
Calcium hydroxide	3.705	$Ca(OH)_2$ = (n.l.t.) 95
Calcium lactate	10.91	$C_6H_{10}CaO_6$ = (n.l.t.) 98
Calcium lactate tablets	15.42	$C_6H_{10}CaO_6.5H_2O$ = 94 to 106[*]
Calcium mandelate	17.12	$C_{16}H_{14}CaO_6$ = (n.l.t.) 98.5
Calcium phosphate, dibasic	8.605	$CaHPO_4.2H_2O$ = (n.l.t.) 98
Cherry juice (malic acid)	6.704	$C_4H_6O_5$ = (n.l.t.)1 *W/V*
Ringer's solution	0.7351[†]	$CaCl_2.2H_2O$ = 0.030 to 0.036 *W/V*
Ringer's solution, lactated	0.7351[†]	$CaCl_2.2H_2O$ = 0.018 to 0.022 *W/V*
N.F.		
Calcium bromide	9.996	$CaBr_2$ = 84 to 94
Calcium carbonate tablets	5.005	$CaCO_3$ = 92.5 to 107.5[*]
Calcium chloride injection	5.550	$CaCl_2.2H_2O$ = 72 to 79
Calcium levulinate	13.52	$C_{10}H_{14}CaO_6$ = 97.5 to 100.5
Calcium levulinate injection	15.32	$C_{10}H_{14}CaO_6.2H_2O$ = 95 to 105[*]
Chalk, prepared	5.005	$CaCO_3$ = (n.l.t.) 97
Sodium bicarbonate and calcium carbonate powder	5.005	$CaCO_3$ = 92.5 to 107.5[*]
Sodium bicarbonate and calcium carbonate tablets	5.005	$CaCO_3$ = 92.5 to 107.5[*]

[*] Per cent of labeled amount.

[†] Titer of 0.01*N* $KMnO_4$.

(n.l.t.) = not less than.

Since each mole of calcium gluconate produces 1 mole or 2 equivalents of calcium oxalate, the equivalent weight of calcium gluconate is 224.2 Gm. or $\frac{1}{2}$ gram-molecular weight. Therefore each cubic centimeter of 0.1N potassium permanganate is equivalent to 0.02242 Gm. or 0.1 meq. of $C_{12}H_{22}CaO_{14}.H_2O$.

This is an indirect method since neither calcium ion nor gluconate ion is measured directly; an equivalent amount of oxalate ion reduces the standard permanganate in the titration. Calculate the $C_{12}H_{22}CaO_{14}.H_2O$ content of the sample and compare the results with the U.S.P. specification.

Questions and Problems

1. Write half equations and combined equations for the reactions involved in the official assay procedures for calcium mandelate and calcium mandelate tablets. Suggest a reason for the differences.

2. The indirect assay procedure for malic acid in cherry juice also involves formation of an equivalent amount of calcium oxalate. Give equations for all reactions and calculate the malic acid titer of 0.1N potassium permanganate.

3. In titration of the solution of CaC_2O_4 obtained from a 0.2600-Gm. sample of calcium chloride, 39.40 cc. of 0.0950N potassium permanganate was required. Calculate the $CaCl_2$ and the $CaCl_2.2H_2O$ content of the sample.

RESIDUAL TITRATION METHODS

The residual titrations in the official assays with potassium permanganate solution are of three types, *viz.:* (1) an excess of standard potassium permanganate solution is employed to oxidize a substance, and the amount in excess is determined by reduction with excess standard oxalic acid solution and back titration with more of the standard permanganate; (2) an excess of standard oxalic acid solution is added to a substance, and the excess of oxalic acid is titrated with standard permanganate solution; (3) an excess of ferrous sulfate solution is added to an oxidizing agent, and the amount of ferrous sulfate in excess is titrated with standard permanganate solution.

The assay of sodium nitrite, page 173, is an example of a residual titration method in which an excess of standard permanganate solution is employed to oxidize a substance, and the amount in excess is determined by reduction with excess standard oxalic acid and back titration with more of the standard permanganate.

Exercise 39

Object. To Prepare and Standardize 0.1*N* Oxalic Acid.

Materials Required. 6.45 Gm. of reagent oxalic acid.
0.1*N* potassium permanganate.

Procedure. Dissolve 6.45 Gm. of reagent oxalic acid in sufficient water to measure 1,000 cc. Ascertain its exact strength by titration against freshly standardized 0.1*N* potassium permanganate and, if desired, adjust to exact normality or to a known strength of approximate normality.

The purest oxalic acid obtainable varies in composition because of differences in its moisture content. If the acid is heated sufficiently to drive off absorbed moisture, it tends to lose water of crystallization. Consequently, a solution of approximate concentration is prepared, and its normality is determined by titration against a standard solution, in this case, 0.1*N* $KMnO_4$. The titration is conducted as follows:

Measure accurately from a burette about 30 cc. of the oxalic acid solution into a beaker and dilute with about 200 cc. of water. Add 7 cc. of sulfuric acid, heat to about 70°, and then slowly add the standard permanganate solution from a glass-stoppered burette, with constant stirring, until a pale pink color is produced which persists for 15 sec. The temperature at the conclusion of the titration should be not less than 60°. Calculate the normality of the solution and, if desired, adjust the solution to exactly 0.1*N*. The calculations and reactions are similar to those described in the preparation of 0.1*N* $KMnO_4$.

The probability of error is minimized by standardizing the oxalic acid solution against the same permanganate solution with which it is to be used in analyses. The normality of an oxalic acid solution may be determined by titration with standard alkali as follows:

Dilute 25 cc. of 0.1*N* NaOH accurately measured from a burette with 25 cc. of distilled water, add 2 to 4 drops of phenolphthalein T.S. as indicator, and heat the solution to boiling. Titrate this solution with 0.1*N* oxalic acid until the pink color disappears. The solution should be brought to a gentle boil at the end to expel all CO_2, which, if present, would affect the phenolphthalein indicator.

The equivalent weight of oxalic acid is the same whether it neutralizes sodium hydroxide or reduces potassium permanganate; hence the same normality would be calculated from data obtained in either of the above methods of standardization.

Oxalic acid solution deteriorates on standing and must be restandardized from time to time.

Questions and Problems

1. Why is hot titration employed?

2. What is the equivalent weight of oxalic acid as an acid; as a reducing agent?

3. Write the reaction involved in the standardization of oxalic acid against sodium hydroxide solution.

4. How much oxalic acid is required to prepare 1,000 cc. of solutions of the following respective normalities: (*a*) 0.5000; (*b*) 0.2500; (*c*) 0.200?

Exercise 40

Object. Assay of Sodium Nitrite.

Materials Required. 1 Gm. of sodium nitrite.
50 cc. of 0.1*N* potassium permanganate.
5 cc. of sulfuric acid.
0.1*N* oxalic acid.

Procedure. 1. "Weigh accurately about 1 Gm. of Sodium Nitrite, previously dried over sulfuric acid for 4 hours, and dissolve it in water to make exactly 100 cc. Add exactly 10 cc. of this solution, from a pipette, to a mixture of 50 cc. of 0.1*N* potassium permanganate, 100 cc. of water, and 5 cc. of sulfuric acid. When adding the Sodium Nitrite solution, immerse the tip of the pipette beneath the surface of the permanganate mixture."

Sodium nitrite is deliquescent and is therefore directed to be dried in a desiccator over sulfuric acid previous to weighing. Because of this property, the sample should be weighed in a stoppered weighing bottle. Nitrous acid is oxidized by the permanganate to nitric acid. If the solution of sodium nitrite were acidified with sulfuric acid, nitrous acid, which is volatile, would be lost. The sodium nitrite is therefore introduced beneath the surface of the acidified permanganate solution.

2. "Warm the liquid to 40°, allow it to stand for 5 minutes, and add 25 cc. of 0.1*N* oxalic acid. Heat the mixture to about 80°, and titrate with 0.1*N* potassium permanganate. Each cubic centimeter of 0.1*N* potassium permanganate is equivalent to 3.450 mg. of $NaNO_2$."

The oxidation of nitrous to nitric acid proceeds slowly at ordinary temperature, but it is rapid at 40°. The warm mixture is allowed to stand for 5 min. to ensure completion of the oxidation. Instead of titrating the excess permanganate with standard oxalic acid to disappearance of the permanganate color, the excess permanganate is reduced by adding an excess of standard oxalic acid and titrating the excess oxalic acid with more of the standard permanganate to appearance of permanganate color. The volume of standard permanganate reduced by the sodium nitrite is then calculated by subtracting it from the sum of the two volumes of permanganate employed and gives the volume reduced by the standard oxalic acid.

$$\underset{69.01}{10NaNO_2} + 4KMnO_4 + 11H_2SO_4 \longrightarrow 10HNO_3 + 4MnSO_4 + 2K_2SO_4 + 5Na_2SO_4 + 6H_2O$$

Each molecule of sodium nitrite loses 2 electrons, and the equivalent weight of sodium nitrite is 34.50 Gm. or ½ gram-molecular weight. Therefore, each cubic centimeter of 0.1N potassium permanganate is equivalent to $0.1 \times 0.0345 = 0.00345$ Gm. or 0.1 meq. of $NaNO_2$.

Sodium nitrite is the only official substance assayed by this method.

Questions and Problems

1. Why is sodium nitrite not assayed by direct titration with standard potassium permanganate in the usual way?

2. A 1.1000-Gm. sample of sodium nitrite was dissolved in sufficient water to make 100 cc. A 10-cc. sample of the solution was added to 50.00 cc. of 0.1N potassium permanganate in presence of sulfuric acid. The mixture was treated with 25.00 cc. of 0.0975N oxalic acid and titrated with 4.58 cc. of 0.1N potassium permanganate. Calculate the per cent $NaNO_2$ in the sample.

The following exercise illustrates methods in which an excess of standard oxalic acid solution is employed to precipitate a substance quantitatively with subsequent residual titration of the excess oxalic acid by standard permanganate solution.

Exercise 41

To Determine the Purity of Lime. Calcium and lead salts can be assayed volumetrically by precipitation with standard oxalic acid added in excess and subsequent titration of the excess acid with standard potassium permanganate solution.

Object. Assay of Lime.

Materials Required. About 1 Gm. of lime.
20 cc. of diluted hydrochloric acid.
50 cc. of 0.1*N* oxalic acid.
Ammonia T.S.
Diluted sulfuric acid.
0.1*N* potassium permanganate.

Procedure. 1. "Ignite about 1 Gm. of Lime to constant weight with a blast lamp, weigh accurately, dissolve in 20 cc. of diluted hydrochloric acid, cool the solution, dilute with water to 100 cc., and mix well."

The N.F. specifies that lime loses not more than 10 per cent of its weight when ignited to constant weight with a blast lamp. Since the moisture content of the material is controlled by the loss on ignition, the objective of the assay procedure is the determination of the proportion of CaO in the freshly ignited material.

2. "Transfer 10 cc. of this solution to a 100-cc. volumetric flask, add 50 cc. of 0.1*N* oxalic acid, render it alkaline with ammonia T.S., shake the mixture well, and allow it to stand for 3 hours at from 60° to 70°, or overnight at room temperature. Cool the liquid, if necessary, fill the flask with water to 100 cc., mix well, filter through a dry filter into a flask, reject the first 10 cc. of the filtrate."

The calcium oxalate, $CaC_2O_4.H_2O$, equivalent to the CaO in a $\frac{1}{10}$ aliquot part of the sample is precipitated by use of standard oxalic acid and ammonia under conditions which promote growth of larger crystals, minimize coprecipitation of oxalates of other metals, and ensure complete precipitation of the calcium ions. Coprecipitation is undesirable in this assay, because subsequent titration with standard permanganate should be a true measure of the oxalic acid in excess of that necessary for complete precipitation of the calcium ions.

The calcium oxalate suspension is diluted to a volume of 100 cc., mixed well, filtered, and the first 10 cc. of filtrate is rejected so that a representative aliquot part of the subsequent filtrate may be measured. This procedure eliminates the tedious washing that would be required to render the calcium oxalate free of excess oxalate ions.

3. "Acidify 50 cc. of the subsequent filtrate with diluted sulfuric acid, then add 25 cc. more of the diluted sulfuric acid, warm the solution to about 70°, and titrate the excess oxalic acid with 0.1*N* potassium permanganate. Each cubic centimeter of 0.1*N* oxalic acid is equivalent to 2.804 mg. of CaO."

The excess oxalic acid in a ½ aliquot part of the filtrate is determined by acidification and titration with standard permanganate in the usual way. The oxalic acid thus found in the aliquot part of the filtrate is one-half of the oxalic acid which was in excess of the amount required for precipitation of the calcium ions in a ⅒ aliquot part of the sample. Corrections for the two aliquot parts used are, of course, necessary in calculation of the CaO content of the sample.

CaO, dissolved in hydrochloric acid, reacts with oxalic acid in presence of ammonia T.S.

$$\underset{56.08}{CaO} + H_2C_2O_4 \longrightarrow CaC_2O_4.H_2O$$

One mole of CaO is equivalent to 1 mole, 2 equivalents, of oxalic acid, and the equivalent weight of CaO is 28.04 Gm. Therefore

TABLE 24. OFFICIAL SUBSTANCES ASSAYED BY RESIDUAL TITRATION OF THE EXCESS OF AN EQUIVALENT AMOUNT OF 0.1*N* OXALIC ACID, USING 0.1*N* $KMnO_4$

Substance	Equivalent of 1 cc. of 0.1*N* oxalic acid	Official requirement, per cent
U.S.P.		
Potassium permanganate	3.161	$KMnO_4$ = (n.l.t.) 99
N.F.		
Lead monoxide	11.16	PbO = (n.l.t.) 97
Lead subacetate solution	10.36	Pb = (n.l.t.) 22.5*W*/*V*
Lead subacetate solution, diluted	10.36	Pb = 0.7 to 0.8*W*/*V*
Lime	2.804	CaO = (n.l.t.) 95
Potassium permanganate tablets	3.161	$KMnO_4$ = 94 to 106*, 92.5 to 107.5†

* Per cent of labeled amount in tablets containing 300 mg. or more.
† Per cent of labeled amount in tablets containing less than 300 mg.
(n.l.t.) = not less than.

each cubic centimeter of 0.1*N* oxalic acid which reacts with CaO to form $CaC_2O_4.H_2O$ is equivalent to $0.1 \times 0.02804 = 0.002804$ Gm. or 0.1 meq. of CaO.

Questions and Problems

1. In the precipitation of $CaC_2O_4.H_2O$ from a solution of CaO in hydrochloric acid, why is the mixture rendered alkaline with ammonia T.S.? What are the sources of error in this precipitation procedure?

2. Will the excess oxalic acid have any effect on the completeness of precipitation of $CaC_2O_4.H_2O$? Explain.

3. If it were desired to measure the total excess of oxalic acid rather than an aliquot part, how would the above procedure be modified?

4. Outline a procedure for analysis of potassium permanganate for $KMnO_4$ which involves use of standard oxalic acid solution and calculate the $KMnO_4$ titer of 0.1*N* oxalic acid. Compare with the U.S.P. assay procedure for potassium permanganate.

5. If potassium permanganate is to be assayed by treatment of a sample with 50.00 cc. of 0.0952*N* oxalic acid, what should be the weight of the permanganate sample (assumed to be 100 per cent $KMnO_4$) so that 10.00 cc. of 0.0915*N* potassium permanganate would be required in the back titration?

6. Write half equations and the combined equation showing the electron transfer that occurs in the assay of lime.

The following assay illustrates a method involving reduction of an oxidizing agent by means of an excess of ferrous sulfate, and residual titration of the excess ferrous sulfate by standard permanganate solution.

Exercise 42

Object. Assay of Reagent Potassium Chlorate.

Materials Required. 0.1 Gm. of potassium chlorate.

100 cc. of acid ferrous sulfate T.S. prepared by dissolving 7 Gm. of ferrous sulfate crystals ($FeSO_4.7H_2O$) in 90 cc. of freshly boiled distilled water and adding sufficient sulfuric acid to make 100 cc.

20 cc. of manganese sulfate T.S. prepared by dissolving 11 Gm. of manganese sulfate ($MnSO_4.4H_2O$) in 50 cc. of distilled water and adding sufficient diluted sulfuric acid to make 100 cc.

0.1*N* potassium permanganate.

Procedure. 1. "Weigh accurately about 100 mg. of potassium chlorate, dissolve it in 10 cc. of water in a 250-cc. flask, and add, from a burette, 35 cc. of acid ferrous sulfate T.S. Prepare a valve-stopper by taking a piece of rubber tubing of convenient diameter and about 5 cm. in length, place a piece of glass rod in one end, and slip the other end over a glass tube which passes through a perforated stopper of a size to fit the flask used. Cut a longitudinal slit about 15 mm. long in one side of the rubber tube about midway of its length. Insert this stopper in the flask and boil the mixture for 10 minutes."

When potassium chlorate is treated with ferrous sulfate T.S. in acid solution, the ferrous salt is oxidized to the ferric state and the potassium chlorate is reduced to potassium chloride:

The half equations

$$ClO_3 + 6H^+ + 6e \longrightarrow Cl^- + 3H_2O$$
$$6Fe^{++} \longrightarrow 6Fe^{3+} + 6e$$

when combined give

$$ClO_3^- + 6Fe^{2+} + 6H^+ \longrightarrow Cl^- + 6Fe^{3+} + 3H_2O$$

and the molecular equation may be written

$$\underset{2(122.55)}{2KClO_3} + 12FeSO_4 + 7H_2SO_4 \longrightarrow K_2SO_4 + 6Fe_2(SO_4)_3 + 2HCl + 6H_2O$$

Sufficient acid must be present to supply an abundance of H^+ to satisfy the equation and to prevent the hydrolysis of the ferric sulfate. If oxygen from the air were allowed to enter, it would oxidize some of the $FeSO_4$ to $Fe_2(SO_4)_3$. The reaction is therefore carried out in a flask fitted with a Bunsen valve, which allows steam to escape but prevents the entrance of air.

2. "Cool the mixture, add 10 cc. of manganese sulfate T.S., and titrate the excess of ferrous sulfate with 0.1*N* potassium permanganate."

The mixture should be cooled in the flask protected from air, and the manganese sulfate solution added just before titration of the residual ferrous sulfate with standard potassium permanganate solution. The presence of manganese sulfate is essential in the titration of Fe^{++} in the presence of Cl^- ions. The manganous ions react with permanganate ions to form manganic ions Mn^{3+}. In this state of oxidation, the manganese is unstable in acid solution, and it is reduced more readily by ferrous ions than by chloride ions.

3. "Conduct a blank test with another portion of 35 cc. of acid ferrous sulfate T.S., measured from a burette, and subtract the result of the former titra-

on from that of the latter. Each cubic centimeter of $0.1N$ potassium permanganate is equivalent to 2.043 mg. of $KClO_3$.”

A blank experiment is conducted employing all the reagents used in the assay but without the addition of potassium chlorate. This procedure is necessary, since the exact concentration of the ferrous ulfate T.S. is not known. The difference between the volumes of $0.1N$ $KMnO_4$ consumed in the blank experiment and in the assay gives the number of cubic centimeters of $0.1N$ $KMnO_4$ equivalent o the sample of potassium chlorate.

The equation above shows that the equivalent weight of $KClO_3$ s 20.43 Gm., $\frac{1}{6}$ gram-molecular weight. Each cubic centimeter of $0.1N$ $KMnO_4$ is equivalent to $0.1 \times 0.02043 = 0.002043$ Gm. or 0.1 meq. of $KClO_3$.

If 49.5 cc. of $0.1N$ $KMnO_4$ was reduced by the ferrous sulfate in the blank, and 4.5 cc. by the excess ferrous sulfate in a test sample containing 0.1000 Gm. of potassium chlorate, then there must have been in the test sample $KClO_3$ equivalent to $49.5 - 4.5 = 45.0$ cc. of $0.1N$ $KMnO_4$, or

$$\frac{45.0 \times 0.002043 \times 100}{0.1} = 91.93 \text{ per cent } KClO_3$$

Questions and Problems

1. If 35.00-cc. portions of acid ferrous sulfate T.S. were used in the blank and test sample in the above example, what was the normality of the acid ferrous sulfate T.S.?
2. The manganese sulfate T.S. is used in the above experiment as a preventive. Give the reason for its presence and its mechanism of action.
3. Why is standard solution of ferrous sulfate not used in the above analytical procedure?

The difficulty in the titration of Fe^{++} in the presence of Cl^-, encountered in the assay of potassium chlorate, and in the presence of sugars in other assays, can be circumvented by use of a standard solution of ceric sulfate as the oxidizing agent. Ceric sulfate is not reduced by Cl^- even in rather high concentrations. A number of official preparations are assayed by cerimetric methods.

Cerimetric Methods. A solution of ceric sulfate in diluted sulfuric acid is a strong oxidizing agent and considerably more stable than standard permanganate solutions provided sufficient sulfuric acid is present to prevent hydrolysis and precipitation of basic salts. Although permanganate can be reduced to any of several oxidation states, Ce^{3+} invariably results on reduction of Ce^{4+}. Solutions of ceric sulfate combine many of the advantages of permanganate and dichromate, *viz:* (1) the solutions are stable even on boiling; (2) they react quantitatively with oxalate or arsenite ion, and either sodium oxalate or arsenic trioxide may be used as a primary standard; (3) the cerous ion is colorless and does not obscure the indicator end point; (4) no intermediate products are formed in the reduction of ceric cerium; (5) rather high concentrations of chloride ion are not oxidized by ceric salts, so that ferrous iron can be determined in the presence of chlorides; (6) the ferrous phenanthroline ion (ferroin) is a very satisfactory indicator in titrations with ceric salts.

Ortho-phenanthroline Test Solution. Dissolve 0.15 Gm. of ortho-phenanthroline in 10 cc. of a solution of ferrous sulfate, prepared by dissolving 1.48 Gm. of clear crystals of ferrous sulfate in 100 cc. of distilled water. The ferrous sulfate solution must be prepared immediately before dissolving the ortho-phenanthroline. Preserve the solution in well-closed containers.

The base ortho-phenanthroline dissolves readily in aqueous solutions of ferrous salts, 3 molecules combining with 1 ferrous ion to form complex ions, known as ferroin, which have an intensely red color. Strong oxidizing agents convert the ferrous to a ferric complex, which has a slight blue color, the intensity of which is much less than that of the ferrous complex, so that it functions practically as a one-color indicator.

$$\underset{\substack{\text{Ortho-phenanthroline}\\ \text{(colorless)}}}{C_{12}H_8N_2} + Fe^{++} \longrightarrow \underset{\substack{\text{Ferrous complex}\\ \text{(red)}}}{Fe(C_{12}H_8N_2)_3^{++}} \rightleftharpoons \underset{\substack{\text{Ferric complex}\\ \text{(blue)}}}{Fe(C_{12}H_8N_2)_3^{+++}} + (-)$$

The color change, red to blue, is reversed by reducing agents. The blue oxidized form is very stable to further change by strong oxidizing agents.

One drop of this indicator solution is equivalent to less than 0.01 cc. of $0.1N$ oxidizing agent, so that the indicator blank is negligible. Stock solutions of the indicator are stable.

Exercise 43

Object. To Prepare and Standardize 0.1*N* Ceric Sulfate.

Materials Required. 42 Gm. of reagent ceric sulfate.
0.2 Gm. of reagent iron wire.
50 cc. of diluted sulfuric acid.
Ortho-phenanthroline test solution.

Procedure. 1. "Dissolve 42 Gm. of reagent ceric sulfate in about 500 cc. f water containing 28 cc. of sulfuric acid, warming if necessary. When soluion is complete, cool, add sufficient water to make 1000 cc., and mix well."

Since the molecular weight of cerium sulfate, $Ce(SO_4)_2$, is 332.25 nd the change of charge from ceric to cerous ion is 1, a 0.1*N* soluion should contain 33.225 Gm. in 1,000 cc. Reagent ceric sulfate ontains variable amounts of water and 42 Gm. in 1,000 cc. ensures ufficient reagent so that the solution may be diluted to exactly 0.1*N*.

2. "Weigh accurately from 170 to 200 mg. of clean, dry reagent iron wire nd transfer to a 250-cc. or a 300-cc. flask. Add 50 cc. of diluted sulfuric acid, lose the flask with a stopper bearing a bunsen valve, and heat on a steam bath ntil the iron is dissolved. Cool the solution, dilute it with 50 cc. of freshly oiled and cooled water, add 2 drops of ortho-phenanthroline T.S., and titrate vith the ceric sulfate solution from a burette until the red color is changed to ale blue. Calculate the normality and, if desired, adjust the solution exactly o 0.1*N*."

The iron wire dissolves in the sulfuric acid and, when the mixture is protected against oxidation by air by means of a Bunsen valve, a solution of an equivalent amount of ferrous sulfate results. The ferrous sulfate reduces the ceric sulfate:

$$\begin{aligned} &Fe \longrightarrow Fe^{++} + 2e \\ &Fe^{++} \longrightarrow Fe^{3+} + e \\ &Ce^{4+} + e \longrightarrow Ce^{3+} \end{aligned}$$

The combined equation may be written $Fe^{++} + Ce^{4+} \longrightarrow Fe^{3+} + Ce^{3+}$ and the molecular equation may be written

$$\underset{151.91}{2FeSO_4} + \underset{332.25}{2Ce(SO_4)_2} \longrightarrow Fe_2(SO_4)_3 + Ce_2(SO_4)_2$$

Each cubic centimeter of a 0.1*N* solution of $Ce(SO_4)_3$ contains 1 meq. or 33.23 mg.

Exercise 44

Object. Assay of Ferrous Carbonate Pills.

Materials Required. 5 ferrous carbonate pills.
20 cc. of diluted sulfuric acid.
0.1*N* ceric sulfate.
Ortho-phenanthroline T.S.

Procedure. "Carefully pulverize 5 Ferrous Carbonate Pills in a mortar, and triturate with 20 cc. of diluted sulfuric acid until all carbonate is dissolved. Transfer completely the contents of the mortar to a beaker of about 800-cc. capacity, and add water to bring the total volume to approximately 300 cc. Add ortho-phenanthroline T.S., and titrate immediately with 0.1*N* ceric sulfate, avoiding excessive stirring. Near the end of the titration tilt the beaker at an angle of 45° to facilitate the detection of the end point. Each cubic centimeter of 0.1*N* ceric sulfate is equivalent to 11.59 mg. of $FeCO_3$."

The ferrous carbonate reacts with the sulfuric acid to form ferrous sulfate,

$$\underset{115.86}{FeCO_3} + H_2SO_4 \longrightarrow FeSO_4 + H_2O + CO_2$$

The ferrous sulfate is oxidized by the ceric sulfate,

$$2FeSO_4 + Ce(SO_4)_2 \longrightarrow Fe_2(SO_4)_3 + Ce_2(SO_4)_3$$

The equivalent weight of ferrous carbonate is 115.86 Gm., 1 gram-molecular weight. Therefore, each cubic centimeter of 0.1*N* ceric sulfate is equivalent to $0.1 \times 0.11586 = 0.011586$ Gm. or 0.1 meq. of $FeCO_3$.

The assay of Menadione U.S.P. is based on the reduction of the quinone, in solution in glacial acetic acid, to the corresponding hydroquinone by means of zinc and hydrochloric acid, and the reoxidation of the hydroquinone to a quinone by ceric sulfate.

The cerimetric procedure for nitrite has been adapted for the assay of the KCl content of Ringer's Solution and Lactated Ringer's olution. The stoichiometry in this procedure can be calculated sing the equations:

$$2KCl + Na_3Co(NO_2)_6 \longrightarrow K_2NaCo(NO_2)_6 + 2NaCl$$

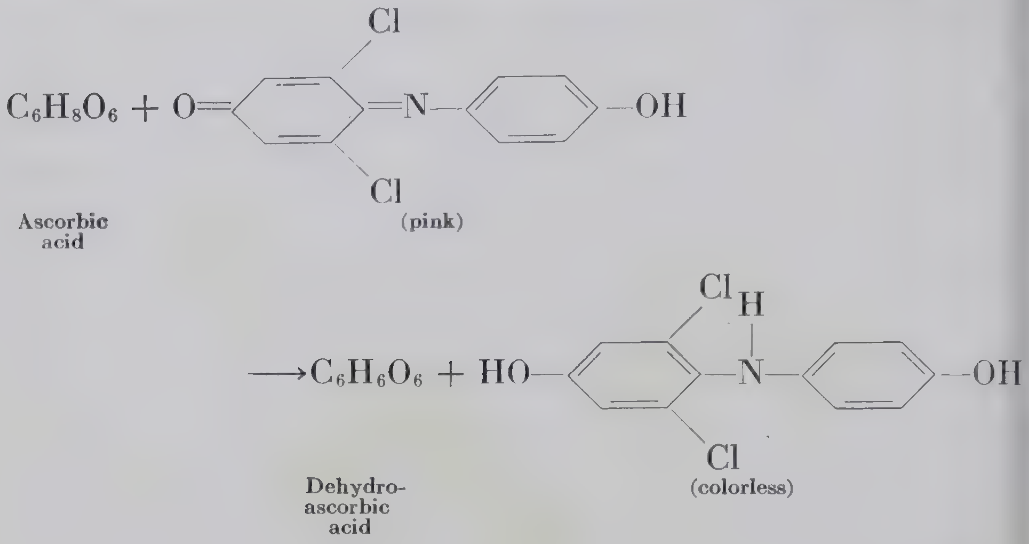
$$K_2NaCo(NO_2)_6 + 12Ce(SO_4)_2 + 6H_2O \longrightarrow Co(NO_3)_3 + 2KNO_3 + NaNO_3 + 6Ce_2(SO_4)_3 + 6H_2SO_4$$

ABLE 25. OFFICIAL ASSAYS BY TITRATION WITH STANDARD CERIC SULFATE

Substance	Normality of $Ce(SO_4)_2$	Equivalent of 1 cc., mg.	Official requirement, per cent
.S.P.			
Ferrous sulfate syrup	**0.1**	**27.80**	**$FeSO_4.7H_2O$ = 3.75 to 4.25 *W/V***
Ferrous sulfate tablets	**0.1**	**27.80**	**$FeSO_4.7H_2O$ = 95 to 110***
Menadione	**0.1**	**8.609**	**$C_{11}H_8O_2$ = (n.l.t.) 98.5**
Menadione tablets	**0.02**	**1.722**	**$C_{11}H_8O_2$ = 95 to 110***
Menadione sodium bisulfite	**0.1**	**13.81**	**$C_{11}H_8O_2.NaHSO_3$ = (n.l.t.) 94**
Menadione sodium bisulfite, injection	**0.02**	**3.303**	**$C_{11}H_8O_2.NaHSO_3.3H_2O$ = 90 to 110***
Ringer's solution	**0.02**	**0.2485**	**KCl = 0.025 to 0.035 *W/V***
Ringer's solution, lactated	**0.02**	**0.2485**	**KCl = 0.027 to 0.033 *W/V***
N.F.			
Ferrous carbonate mass	**0.1**	**11.59**	**$FeCO_3$ = 36 to 41**
Ferrous carbonate pills	**0.1**	**11.59**	**$FeCO_3$ = 60 mg. per pill**
Ferrous carbonate, saccharated	**0.1**	**11.59**	**$FeCO_3$ = (n.l.t.) 15**
Ferrous gluconate	**0.1**	**5.585**	**Fe = (n.l.t.) 11.5**
Iron, reduced	**0.1**	**5.585**	**Fe = (n.l.t.) 90**
Iron, reduced, capsules	**0.1**	**5.585**	**Fe = 86 to 102***

*** Per cent of labeled amount.**
(n.l.t.) = not less than.

Assay of Ascorbic Acid. A solution of dichlorophenol-indophenol, which has been standardized using U.S.P. ascorbic acid reference standard, is used as a standard oxidizing agent in determination of the ascorbic acid content of ascorbic acid tablets, sodium ascorbate injection, hexavitamin capsules, and hexavitamin tablets. No indicator is necessary since the oxidized form of the dichlorophenol-indophenol is colored blue in alkali and red in acid while the reduced form is colorless. A solution of ascorbic acid from the sample is titrated in the presence of metaphosphoric acid and acetic acid with the dichlorophenol-indophenol standard solution until a distinct rose-pink color, persisting for at least 5 sec., results:


$C_6H_8O_6$ + O=
Cl
N
OH
Cl
Ascorbic acid
(pink)
→$C_6H_6O_6$ + HO—
Cl
H
N
OH
Cl
Dehydroascorbic acid
(colorless)

Chapter 9

OXIDATION AND REDUCTION. IODIMETRIC AND IODOMETRIC METHODS

The reversible reaction $2I^- \rightleftharpoons I_2 + 2e$ can be applied in analysis of reducing agents such as thiosulfates and arsenites by the use of standard solutions of iodine. Such a direct procedure is sometimes designated as *iodimetry*. The same reversible reaction can be applied indirectly in the analysis of oxidizing agents such as ferric and cupric salts, and "available" chlorine. In the indirect procedure, known as *iodometry*, the sample of oxidizing agent is reduced with excess potassium iodide and an equivalent amount of iodine is produced. The iodine thus formed is titrated with a standard solution of sodium thiosulfate.

Iodometric methods include some of the most accurate procedures in titrimetric analysis because, under proper conditions, the presence of 1 part of iodine in several million parts of solution is readily detected by use of starch indicator solution. In some procedures, the color of iodine itself may be used in observing the end point of a titration, especially when a small volume of carbon tetrachloride or carbon disulfide, in which iodine is soluble, is added to the mixture. The iodine which is concentrated in the immiscible solvent layer imparts a distinct color to it, even when the total amount of iodine in the mixture is very small.

$$I_2 + \underset{\substack{\text{Sodium}\\\text{thiosulfate}}}{2Na_2S_2O_3} \longrightarrow 2NaI + \underset{\substack{\text{Sodium}\\\text{tetrathionate}}}{Na_2S_4O_6}$$

This equation shows that 1 gram-atomic weight of iodine, 126.92 Gm., and 1 gram-molecular weight of sodium thiosulfate, $Na_2S_2O_3 \cdot 5H_2O$, 248.21 Gm., are equivalent.

Iodine is not very soluble in water but dissolves readily in presence of potassium iodide because of the formation of the tri-iodide ion

$I_2 + I^- \longrightarrow I_3^-$. For this reason standard iodine solutions used in iodimetry are solutions of iodine in potassium iodide solution. Moreover, excess potassium iodide is used as a reducing agent in iodometry so that the iodine formed will remain in solution through interaction with potassium iodide.

In alkaline solutions, reaction of iodine with OH^- produces first hypoiodite, and finally iodate, ions.

$$I_2 + OH^- \longrightarrow HI + IO^-$$
$$3IO^- \longrightarrow IO_3^- + 2I^-$$

These ions oxidize thiosulfate, at least partially, to a higher oxidation state, *e.g.*, sulfate, and the stoichiometry indicated above no longer holds true. Consequently, iodometric procedures are never carried out in strongly basic solutions.

The uniformity of the reaction of iodine with sodium thiosulfate accounts for the use of standard solution of iodine in the analysis of sodium thiosulfate and the use of standard solution of sodium thiosulfate in the titrimetric determination of iodine in various iodimetric and iodometric procedures.

Iodine quantitatively oxidizes certain other reducing agents, and standard solutions of iodine may be used in their direct titrimetric analysis, *e.g.*, arsenious acid.

$$H_3AsO_3 + H_2O + I_2 \rightleftharpoons H_3AsO_4 + 2H^+ + 2I^-$$

If a residual method of iodimetry is employed, the excess iodine may be titrated with standard sodium thiosulfate, *e.g.*, the assay of mercurous chloride.

$$2HgCl + I_2 + 6KI \longrightarrow 2K_2HgI_4 + 2KCl$$
$$I_2 + 2Na_2S_2O_3 \longrightarrow 2NaI + Na_2S_4O_6$$

In the iodometric analysis of oxidizing agents, an equivalent amount of iodine is produced when the sample of the oxidizing agent oxidizes potassium iodide in presence of an acid, *e.g.*, assay of ferric chloride.

$$2FeCl_3 + 2KI \longrightarrow 2FeCl_2 + I_2 + 2KCl$$

The equivalent amount of iodine formed in this way can be measured by titration with standard sodium thiosulfate. When potassium iodide is oxidized in strongly acid solution, care must be taken to avoid simultaneous oxidation of the iodide by atmospheric oxygen; otherwise values which are too high will be obtained.

Starch Indicator Solution. This solution is prepared as follows:

Triturate 1 Gm. of arrowroot starch with 10 cc. of cold water in a mortar to form a thin paste. Heat about 200 cc. of water to boiling and add the starch paste to it with constant stirring. Boil the mixture gently for about 30 min. or until it forms a thin translucent liquid. Since a solution of starch deteriorates rapidly, it should be prepared freshly each day.

In hot water, starch granules burst and form a colloidal dispersion of β-amylose, known as soluble starch, and should be separated by decantation from α-amylose, amylopectin, which is insoluble.

Interaction of iodine and iodide ion with the colloidal β-amylose results in the production of an intensely blue-colored sol, and this color change is reversible, the color being discharged when the iodine is reduced with sodium thiosulfate or other reducing agent. The sensitivity of the indicator is greater in slightly acid medium and is markedly decreased by temperatures above 25°, strong solutions of electrolytes, alcohol, and other organic solvents. The reversibility of color formation is decreased when the iodine concentration is high. For this reason the indicator should not be added in residual iodimetric procedures or in iodometric procedures until most of the iodine has been reduced, as shown by the change from a brown to straw-colored solution.

Standard Solutions. The standard solutions employed in the official assays by iodometric methods are 0.1*N* iodine and 0.1*N* sodium thiosulfate.

Exercise 45

Object. To Prepare and Standardize 0.1*N* Sodium Thiosulfate.

Materials Required. 26 Gm. of sodium thiosulfate ($Na_2S_2O_3.5H_2O$).
0.2 Gm. of sodium carbonate.
2 Gm. of potassium iodide.
5 cc. of hydrochloric acid.

0.1*N* potassium dichromate.
Starch indicator solution.

Procedure. 1. "Dissolve about 26 Gm. of sodium thiosulfate and 200 mg. of sodium carbonate in 1000 cc. of recently boiled and cooled water."

The thiosulfate is dissolved in water which has been recently boiled primarily for purposes of sterilization since deterioration of the solution results mainly from the action of bacteria. Boiling also expels carbon dioxide which, if present as carbonic acid, may act like any other acid in causing hydrolysis and decomposition of the thiosulfate.

$$Na_2S_2O_3 + 2H_2CO_3 \longrightarrow 2NaHCO_3 + H_2S_2O_3$$
$$H_2S_2O_3 \longrightarrow H_2SO_3 + S$$

The sodium bicarbonate is added to the solution as a preservative to prevent acid catalyzed hydrolysis.

2. "Measure accurately 30 cc. of 0.1*N* potassium dichromate into a glass-stoppered flask and dilute it with 50 cc. of water. Add 2 Gm. of potassium iodide and 5 cc. of hydrochloric acid, stopper and allow to stand for 10 minutes. Dilute with 100 cc. of water and titrate the liberated iodine with the sodium thiosulfate solution. When the solution has assumed a yellowish-green color, add starch T.S. and continue with the titration to the discharge of the blue color. Calculate the normality of the sodium thiosulfate solution and, if desired, adjust exactly to 0.1*N*."

The potassium dichromate oxidizes iodide ion in the presence of acid to an equivalent amount of iodine, and chromic acid is reduced to the green chromic chloride.

The half equations

$$Cr_2O_7^{--} + 14H^+ + 6e \longrightarrow 2Cr^{3+} + 7H_2O$$
$$6I^- \longrightarrow 3I_2 + 6e$$

are the basis for the molecular equation

$$K_2Cr_2O_7 + 6KI + 14HCl \longrightarrow 3I_2 + 2CrCl_3 + 8KCl + 7H_2O$$

The iodine formed in this reaction will reduce a chemically equivalent amount of sodium thiosulfate. In other words, the volume of sodium thiosulfate used in titration of the iodine is chemically equivalent to the 30.00 cc. of 0.1 *N* potassium dichromate. Therefore, the normality of the sodium thiosulfate solution may be calculated:

$$\frac{30 \times 0.1}{\text{cc. of } Na_2S_2O_3} = \text{normality of the } Na_2S_2O_3 \text{ solution}$$

Potassium iodide in excess of the amount necessary for reduction of the $K_2Cr_2O_7$ is employed so that the liberated iodine will be held in solution as KI_3. The solution is diluted before titration to make the end point more readily observed since the mixture does not become colorless when all the iodine is reduced but rather changes from the deep blue color of starch-iodide-iodine complex to the light green Cr^{3+}.

The change in the titer of sodium thiosulfate solutions on standing results primarily from bacterial action. The pH of the solution prepared as above directed using sodium carbonate is unfavorable to the growth of the bacteria in question; but, as a further precaution, specially cleaned glassware and freshly boiled water should be used in preparation of the solution and subsequent gross bacterial contamination of the solution should be avoided.

Sodium thiosulfate solution may also be standardized against pure iodine dissolved in potassium iodide solution or against iodine set free from acidified solution of potassium iodide by standard solutions of potassium permanganate or various primary standard oxidizing agents such as potassium dichromate, potassium iodate, etc.

Questions and Problems

1. Calculate the titer values of each of the following substances for 0.1*N* sodium thiosulfate and the size of the sample of each that would liberate sufficient iodine to consume 35 cc. of 0.1*N* sodium thiosulfate in a titration: (*a*) $K_2Cr_2O_7$; (*b*) KIO_3; (*c*) 0.1*N* $KMnO_4$.

2. What is the iodine titer of 0.1*N* sodium thiosulfate? If all the $Na_2S_2O_3$ of the 0.1*N* solution were converted to Na_2SO_4 in accordance with the equations given, what would be the iodine titer of the resulting solution?

3. In the procedure for standardization of the sodium thiosulfate solution, 30 cc. of 0.1*N* potassium dichromate is employed. Calculate the theoretical amount of KI necessary for reduction of the $K_2Cr_2O_7$ and compare this value with the 2 Gm. of potassium iodide actually used. Account for the discrepancy.

4. Why do sodium thiosulfate solutions undergo a change in titer, and what measures are taken to prevent this?

Exercise 46

Object. To Prepare 0.1*N* Iodine Solution.

Materials Required. 13 Gm. of pure iodine.
36 Gm. of potassium iodide.

Procedure. "Weigh accurately about 12.75 Gm. of reagent iodine and transfer it quickly into a solution of 36 Gm. of potassium iodide in 100 cc. of water. After solution is complete, add 3 drops of hydrochloric acid and dilute to exactly 1000 cc. at 25°. From the weight of the iodine used calculate the normality. The normality of the solution should frequently be redetermined."

One gram of iodine is soluble in 2,950 cc. of water. In the presence of sufficient potassium iodide, however, its solubility is greatly increased because of the formation of the complex I_3^- anion:

$$KI + I_2 \rightleftharpoons KI_3$$

In the presence of reducing agents, the reaction proceeds quantitatively to the left, and the solution reacts like a solution of iodine alone.

The iodine should be weighed accurately in a glass-stoppered weighing bottle, transferred quantitatively to the solution of 36 Gm. of KI in 100 cc. of distilled water; and, after it is completely dissolved, the solution should be made up to exactly 1,000 cc. The solution of potassium iodide used to dissolve the iodine is of sufficient concentration to dissolve the iodine rapidly so that no appreciable loss will occur because of volatilization. If pure reagent iodine is used in preparing this solution, it will be approximately 0.1*N* and need not be standardized. However, because of the difficulty of weighing exact quantities of iodine, it is usually more convenient to prepare a solution of approximate strength and standardize it against 0.1*N* sodium thiosulfate solution as directed in the following exercise.

Exercise 47

Object. To Prepare 0.1*N* Iodine and Standardize It against Sodium Thiosulfate.

Materials Required. 14 Gm. of pure iodine.
36 Gm. of potassium iodide.

$0.1N$ sodium thiosulfate.
Starch test solution.

Procedure. Dissolve about 14 Gm. of reagent iodine in a solution of 36 Gm. of potassium iodide in 100 cc. of water, add 3 drops of hydrochloric acid, diluting finally to 1,000 cc. Carefully measure 25 cc. of this solution into a flask, then add gradually and cautiously from a burette $0.1N$ sodium thiosulfate, shaking constantly, until the color of the solution is but slightly yellow. Then add about 3 cc. of starch T.S. and continue the addition of the $0.1N$ sodium thiosulfate until the blue color is just discharged. Note the number of cubic centimeters of the $0.1N$ sodium thiosulfate consumed, and then dilute the iodine solution so that 25 cc. will require for decolorization exactly 25 cc. of the $0.1N$ sodium thiosulfate at standard temperature.

Although the U.S.P. directs that reagent iodine be used in preparing $0.1N$ iodine solution, iodine containing chlorine in small amounts as impurity could be used, since the chlorine reacts with potassium iodide to liberate iodine:

$$ICl + KI \rightleftharpoons KCl + I_2$$

The sodium thiosulfate reacts with the iodine to form sodium iodide and sodium tetrathionate, and a very slight excess of thiosulfate discharges the blue color of the indicator:

$$I_2 + 2Na_2S_2O_3 \rightleftharpoons 2NaI + Na_2S_4O_6$$

If 25 cc. of approximately $0.1N$ iodine require exactly 25 cc. of $0.1N$ sodium thiosulfate, the iodine is exactly $0.1N$, but, if only 24 cc. of $0.1N$ iodine solution are required, the solution is stronger than normal and each 24 cc. of iodine solution must be diluted to 25 cc. to secure an exactly $0.1N$ solution. Since $0.1N$ iodine solution deteriorates, it will not remain precisely $0.1N$ very long. It is therefore best to calculate the normality of the solution and employ it as a standard solution of known value. In the above case, the approximately $0.1N$ iodine solution would be $25/24 = 1.0417$ times $0.1N$ and should be labeled $0.1042N$ iodine. Whenever the solution is used in a determination, the number of cubic centimeters of $0.1N$ odine employed may be calculated; *i.e.*, cubic centimeters of approximately $0.1N$ iodine $\times$ 1.0417 = cubic centimeters $0.1N$ iodine.

Iodine solutions may also be standardized using pure arsenic trioxide as a primary standard (see Exercise 49).

Questions and Problems

1. Why may iodine containing chlorine or bromine as impurity be used to prepare standard solutions of iodine when the solution is standardized against sodium thiosulfate?
2. What is the normality of a solution containing 0.0136 Gm. of iodine per cubic centimeter?
3. What weight of arsenic trioxide (98.73 per cent As_2O_3) would be used as sample so that 35.00 cc. of 0.1*N* iodine would be required in a titration?
4. A solution of iodine has an iodine titer of 11.43 mg.; what is its As_2O_3 titer?
5. Why is starch T.S. not added during the titration until the solution is but slightly yellow?

OXIDATION-REDUCTION METHODS BY DIRECT TITRATION WITH STANDARD IODINE SOLUTION

Exercise 48

Object. Assay of Arsenic Trioxide.

Materials Required. About 0.2 Gm. of arsenic trioxide.

Sodium hydroxide test solution prepared by dissolving 4.3 Gm. of NaOH in sufficient water to make 100 cc.

Diluted sulfuric acid.

2 Gm. of sodium bicarbonate.

0.1*N* iodine.

Starch indicator solution.

Procedure. 1. "Dissolve about 200 mg. of Arsenic Trioxide, previously dried at 105° for 2 hours and accurately weighed, in 20 cc. of boiling water, adding sufficient sodium hydroxide T.S. to effect complete solution."

Arsenic trioxide is slowly soluble in cold water, more rapidly soluble in boiling water, and readily soluble in sodium hydroxide solution with the formation of sodium arsenite:

$$\underset{197.82}{As_2O_3} + 6NaOH \rightleftharpoons 2Na_3AsO_3 + 3H_2O$$

Sodium arsenite on hydrolysis yields sodium hydroxide and arsenious compounds to form a distinctly alkaline solution.

2. "Neutralize this solution with diluted sulfuric acid, using phenolphthalein T.S. as the indicator, cool, dissolve in it 2 Gm. of sodium bicarbonate, and titrate with 0.1*N* iodine, using starch T.S. as the indicator. Each cubic centimeter of 0.1*N* iodine is equivalent to 4.946 mg. of As_2O_3."

If iodine is added to this alkaline solution, some of it tends to form sodium hypoiodite, NaIO, or similar compounds, which do not react readily with arsenious acid:

$$2NaOH + I_2 \longrightarrow NaIO + NaI + H_2O$$

The excess sodium hydroxide, therefore, required to dissolve the As_2O_3 is exactly neutralized by sulfuric acid. Sodium bicarbonate is added to neutralize the HI formed in the reversible reaction

$$H_3AsO_3 + H_2O + I_2 \rightleftharpoons H_3AsO_4 + 2HI$$

The $NaHCO_3$ removes the HI as rapidly as it is formed, causing the reaction to go to completion toward the right:

$$H_3AsO_3 + I_2 + 2NaHCO_3 \longrightarrow H_3AsO_4 + 2NaI + 2CO_2 + H_2O$$

Under the conditions of the assay, sodium bicarbonate does not react with iodine, but, if an excess of iodine is added, it may react slightly. The reaction between arsenious acid and iodine proceeds quantitatively to completion best in a neutral solution. Since HI is formed in the reaction, a buffer must be added to keep the solution neutral. The sodium bicarbonate in this case is the buffer.

Each mole of As_2O_3 is equivalent to 2 moles or 4 equivalents of H_3AsO_3; therefore the equivalent weight of As_2O_3 is 49.46 Gm., ¼ gram-molecular weight. Consequently, each cubic centimeter of 0.1*N* iodine is equivalent to $0.1 \times 0.04946 = 0.004946$ Gm. or 0.1 meq. of As_2O_3.

Arsenic trioxide, N.F., should contain not less than 99.5 per cent of As_2O_3. Calculate percentage purity of the As_2O_3 assayed and the per cent of arsenic that it contains. Many arsenious compounds, antimonous compounds, etc., and their preparations may be assayed by a similar procedure.

Questions and Problems

1. Summarize the equations for the reactions involved in the assay of arsenic trioxide, in a single equation which shows the chemical equivalence of 1 mole of As_2O_3 and 2 moles or 4 equivalents of iodine.

2. Why is the solution buffered at a pH slightly greater than 7 before titration with iodine? How is the buffering accomplished?

3. Calculate the equivalent weights and 0.1*N* iodine titer values for each of the following and check the calculated values with those given in the U.S.P. and N.F.: (*a*) antimony potassium tartrate ($C_4H_4KO_7Sb.\frac{1}{2}H_2O$); (*b*) ascorbic acid ($C_6H_8O_6$); (*c*) sodium cacodylate ($C_2H_6AsNaO_2$); (*d*) dichlorophenarsine hydrochloride.

4. Outline the steps in the assay of Carbarsone indicating the chemical changes involved in each step. How could this procedure be adapted for analysis of an inorganic pentavalent arsenical such as sodium arsenate?

TABLE 26. OFFICIAL SUBSTANCES ASSAYED BY TITRATION WITH 0.1*N* IODINE

Substance	Equivalent to 1 cc., mg.	Official requirement, per cent
U.S.P.		
Antimony potassium tartrate	16.70	$C_4H_4KO_7Sb.\frac{1}{2}H_2O$ = (n.l.t.) 99
Ascorbic acid	8.806	$C_6H_8O_6$ = (n.l.t.) 99
Carbarsone	13.00	$C_7H_9AsN_2O_4$ = 97.5 to 101
Carbarsone capsules	13.00	$C_7H_9AsN_2O_4$ = 92 to 108*
Carbarsone tablets	13.00	$C_7H_9AsN_2O_4$ = 93 to 107*
Dichlorophenarsine hydrochloride	3.746	As, trivalent = 25 to 27
Dimercaprol	6.211	$C_3H_8OS_2$ = (n.l.t.) 99
Dimercaprol injection	6.211	$C_3H_8OS_2$ = 9 to 11
Oxophenarsine hydrochloride	3.746	As, trivalent = 29.5 to 32
Penicillin G potassium	0.414†	Total penicillins
Penicillin G sodium	0.397†	Total penicillins
Stibophen	38.46	$C_{12}H_4Na_5O_{16}S_4Sb$ = 98.5 to 102
Stibophen injection	44.76	$C_{12}H_4Na_5O_{16}S_4Sb.7H_2O$ = 94 to 106*
Tryparsamide	14.80	$C_8H_{10}AsN_2NaO_4$ = 99 to 101
N.F.		
Arsenic trioxide	4.946	As_2O_3 = (n.l.t.) 99.5
Arsenious acid solution	4.946	As_2O_3 = 0.975 to 1.025 W/V
Ferric cacodylate	3.746	As = 41 to 45
Ferric cacodylate injection	3.746	As = 38.7 to 47.3‡
Potassium arsenite solution	4.946	As_2O_3 = 0.95 to 1.05 W/V
Sodium cacodylate	7.999	$C_2H_6AsNaO_2$ = 72 to 75
Sodium cacodylate injection	7.999	$C_2H_6AsNaO_2$ = 71 to 77§
Sodium thiosulfate	15.81	$Na_2S_2O_3$ = (n.l.t.) 99
Sodium thiosulfate injection	15.81	$Na_2S_2O_3$ = 61 to 67¶

* Per cent of labeled amount.

† 0.01*N* iodine used.

‡ Per cent of labeled amount of $C_6H_{18}As_3FeO_6$.

§ Per cent of labeled amount of $C_2H_6AsNaO_2.3H_2O$.

¶ Per cent of labeled amount of $Na_2S_2O_3.5H_2O$.

(n.l.t.) = not less than.

5. Consult the original literature (Alicino, *Ind. Eng. Chem.*, Anal. Ed., **18**, 619, 1946) and explain how the Penicillin G sodium titer value for 0.01*N* iodine was obtained.

6. What sample weight of each of the following would be used so that 35 cc. of 0.1*N* iodine would be consumed in the titration? Compare with the sample weights specified in U.S.P. and N.F. assay procedures. (*a*) Sodium thiosulfate; (*b*) stibophen; (*c*) tryparsamide; (*d*) sodium cacodylate.

Iodine and preparations containing iodine may be assayed by direct titration with 0.1*N* sodium thiosulfate or 0.1*N* potassium arsenite. The assay of Iodine, U.S.P., using 0.1*N* sodium thiosulfate is accomplished by a procedure which is quite similar to that used for standardizing 0.1*N* iodine solution (Exercise 46). The use of standard potassium arsenite solution for the titrimetric analysis of the iodine content of a preparation is illustrated by the procedure for Strong Iodine Solution.

Exercise 49

Object. To Prepare and Standardize 0.1*N* Potassium Arsenite.

Materials Required. 5 Gm. of reagent potassium arsenite.

Procedure. "Dissolve 4.9455 Gm. of reagent arsenic trioxide, which has been pulverized and dried to constant weight at 100°, in 75 cc. of normal potassium hydroxide. Add 40 Gm. of potassium bicarbonate dissolved in about 200 cc. of water, and dilute with water to exactly 1000 cc. at 25°."

Arsenic trioxide dissolves on reaction with potassium bicarbonate to produce a solution of an equivalent amount of potassium metaarsenite.

$$\underset{197.82}{As_2O_3} + 2KHCO_3 \longrightarrow 2KAsO_2 + H_2O + 2CO_2$$

When used as a reducing agent in the titration of iodine, the metaarsenite is oxidized to arsenate as follows:

$$HAsO_2 + 2H_2O \longrightarrow H_3AsO_4 + 2H^+ + 2e$$

The molecular equation for the titration may be written

$$KAsO_2 + I_2 + 2H_2O \longrightarrow KH_2AsO_4 + 2HI$$

A summation of the equations shows the chemical equivalence of 1 mole of As_2O_3, 2 moles of $KAsO_2$, and 2 moles or 4 equivalents of iodine. Consequently the equivalent weight of As_2O_3 is 49.455 Gm., and in 1,000 cc. of 0.1*N* potassium arsenite, $0.1 \times 49.455 = 4.9455$ Gm. of arsenic trioxide is required.

Exercise 50

Object. Assay of Strong Iodine Solution.

Materials Required. 5 cc. of strong iodine solution.
0.1*N* potassium arsenite.
Starch indicator solution.

Procedure. "Place exactly 5 cc. of Strong Iodine Solution in a 500-cc. glass-stoppered flask, add 25 cc. of water, and titrate with 0.1*N* potassium arsenite, using starch T.S. as the indicator. Each cubic centimeter of 0.1*N* potassium arsenite is equivalent to 12.69 mg. of I."

The explanation for the procedure and the reactions is similar to that given for the assay of arsenic trioxide, page 192.

TABLE 27. OFFICIAL SUBSTANCES ASSAYED BY DIRECT TITRATION WITH 0.1*N* $KAsO_2$

Substance	Equivalent of 1 cc., mg.	Official requirement, per cent
U.S.P.		
Iodine solution, strong	12.69	I = 4.5 to 5.5*W/V*
Iodine tincture	12.69	I = 1.8 to 2.2*W/V*
N.F.		
Iodine ampuls	12.69	I = 1.8 to 2.2*W/V*
Iodine solution	12.69	I = 1.8 to 2.2*W/V*
Iodine tincture, strong	12.69	I = 6.8 to 7.5*W/V*

OXIDATION-REDUCTION METHODS INVOLVING THE USE OF EXCESS STANDARD IODINE SOLUTION AND RESIDUAL TITRATION OF THE EXCESS IODINE WITH STANDARD SODIUM THIOSULFATE SOLUTION

Exercise 51

Object. Assay of Mild Mercurous Chloride.

Materials Required. About 0.7 Gm. of mild mercurous chloride.
50 cc. of 0.1*N* iodine.
4 Gm. of potassium iodide.

0.1N sodium thiosulfate.
Starch indicator solution.

Procedure. 1. "Dry about 700 mg. of Mild Mercurous Chloride over sulfuric acid for 5 hours, weigh accurately, mix well with 10 cc. of water in a glass-stoppered flask, and add 50 cc. of 0.1N iodine and 1 Gm. of potassium iodide dissolved in 10 cc. of water."

Since mercurous chloride is practically insoluble in water, the purity of the salt cannot be determined by the precipitation method used in determination of the chloride ion in a soluble chloride. When mercurous chloride is treated with a solution of iodine in potassium iodide, it dissolves quite readily with the formation of a double salt of mercuric and potassium iodides:

TABLE 28. OFFICIAL SUBSTANCES ASSAYED BY RESIDUAL TITRATION OF EXCESS 0.1N IODINE WITH 0.1N SODIUM THIOSULFATE

Substance	Equivalent of 1 cc., mg.	Official requirement, per cent
U.S.P.		
Sodium bisulfite	5.204	$NaHSO_3$ = (n.l.t.) 95
N.F.		
Mercurous chloride, mild	23.61	HgCl = (n.l.t.) 99.6
Mercurous chloride, mild, and sodium bicarbonate tablets	23.61	HgCl = 92.5 to 107.5,* 90 to 110†
Mercurous chloride, mild, tablets	23.61	HgCl = 92.5 to 107.5‡
Mercurous iodide, yellow	32.75	HgI = (n.l.t.) 99
Santonin and mild mercurous chloride tablets	23.61	HgCl = 92.5 to 107.5‡
Sodium sulfite, exsiccated	6.303	Na_2SO_3 = (n.l.t.) 95

* Per cent of labeled amount in tablets containing more than 15 mg.
† Per cent of labeled amount in tablets containing 15 mg. or less.
‡ Per cent of labeled amount.
(n.l.t.) = not less than.

$$\underset{2(236.07)}{2HgCl} + 6KI + I_2 \longrightarrow 2HgK_2I_4 + 2KCl$$

2. "Stopper the flask, allow the mixture to stand with occasional agitation until complete solution takes place, and then titrate the excess iodine with 0.1N sodium thiosulfate, using starch T.S. as the indicator. Each cubic centimeter of 0.1N iodine is equivalent to 23.61 mg. of HgCl."

In the assay process, the mercurous chloride mixture is allowed to stand with occasional agitation until the reaction has gone to completion as indicated by its complete solution. The excess 0.1N iodine added is then determined by residual titration. The formula of the double salt of mercuric potassium iodide may be written $HgI_2.2KI$, and the ionic reaction may be represented as

$$2Hg^{+} + I_2 \longrightarrow 2Hg^{++} + 2I^{-}$$

Since each molecule of mercurous chloride loses 1 electron on oxidation, its equivalent weight is 236.07 Gm., 1 gram-molecular weight. Each cubic centimeter of 0.1N iodine reduced by the mercurous chloride in the sample is equivalent to $0.1 \times 0.23607 = 0.02361$ Gm. or 0.1 meq. of HgCl.

Questions and Problems

1. Why is it impracticable to assay the following substances by direct titration with standard iodine solution: (*a*) mild mercurous chloride; (*b*) sodium bisulfite?

2. Give equations for the reaction of iodine with $NaHSO_3$ and Na_2SO_3, calculate the weight of each of these substances that is equivalent to 1 cc. of 0.1N iodine, and compare the values with those given in the official assay procedures.

3. A 0.6500-Gm. sample of mild mercurous chloride was treated with 50.00 cc. of 0.1045N iodine and excess potassium iodide. The resulting solution was titrated with 26.24 cc. of 0.0985N sodium thiosulfate. Calculate the per cent HgCl in the sample.

4. Acetone may be assayed by treatment with excess standard iodine solution in presence of a base and titration of the excess iodine with standard sodium thiosulfate. Give equations, calculate the equivalent weight of acetone and prepare an outline of a procedure specifying exact quantities. Compare the latter with the procedure given in N.F. VIII.

OXIDATION-REDUCTION METHODS INVOLVING THE LIBERATION OF IODINE FROM POTASSIUM IODIDE AND TITRATION OF THE FREE IODINE WITH STANDARD SODIUM THIOSULFATE SOLUTION

Exercise 52

Object. Assay of Ferric Chloride Solution.

Materials Required. About 2 Gm. of ferric chloride solution.
5 cc. of hydrochloric acid.

3 Gm. of potassium iodide.
0.1N sodium thiosulfate.
Starch indicator solution.

Procedure. 1. "Transfer about 2 Gm. of Ferric Chloride Solution to a tared flask, stopper, weigh accurately, and add 5 cc. of hydrochloric acid, 25 cc. of water, and about 3 Gm. of potassium iodide. Allow the mixture to stand during 15 minutes."

The mixture is allowed to stand to permit the reduction of ferric chloride and production of an equivalent amount of iodine,

$$\underset{2(162.22)}{2FeCl_3} + 2KI \longrightarrow 2FeCl_2 + I_2 + 2KCl$$

or

$$2Fe^{3+} + 2I^- \longrightarrow 2Fe^{++} + I_2$$

The iodine thus formed is held in solution by the excess potassium iodide.

2. "Dilute it with 100 cc. of water, and titrate the liberated iodine with 0.1N sodium thiosulfate, using starch T.S. as the indicator. Each cubic centimeter of 0.1N sodium thiosulfate is equivalent to 5.585 mg. of Fe."

The equation reveals that 1 mole of $FeCl_3$ is 1 equivalent; therefore, since 1 mole of $FeCl_3$ contains 1 gram-atomic weight of Fe, the equivalent weight of Fe in this procedure is 55.85 Gm., 1 gram-atomic weight. Each cubic centimeter of 0.1N sodium thiosulfate is equivalent to $0.1 \times 0.05585 = 0.005585$ Gm. or 0.1 meq. of Fe.

According to the N.F. definition, Ferric Chloride Solution should contain not less than 10 per cent or more than 11 per cent of Fe. Calculate the per cent of Fe and the per cent of $FeCl_3$ in the sample assayed and compare the results obtained with the official requirement.

A considerable number of official preparations of iron, ferric oxide, and iron salts are assayed by this iodometric procedure (Table 29). In several of these procedures, after the sample has been brought into solution using hydrochloric acid, hydrogen peroxide is added to oxidize all the iron to the Fe^{3+} state, and the excess hydrogen peroxide is removed by evaporation before adding the potassium iodide.

Questions and Problems

1. What preliminary treatment of the sample is necessary in the assay of each of the following substances before the iron content may be determined iodometrically? (*a*) peptonized iron; (*b*) peptonized iron and manganese solution; (*c*) yellow ferric oxide; (*d*) ferric cacodylate; (*e*) beef, iron, and wine.

2. Calculate the Fe content of ferric chloride solution, a 2.1500-Gm. sample of which was treated with potassium iodide, producing a mixture which consumed 37.15 cc. of sodium thiosulfate solution in a titration. Each cubic centimeter of the sodium thiosulfate solution was equivalent to 0.00725 Gm. of pure $K_2Cr_2O_7$.

TABLE 29. OFFICIAL SUBSTANCES ASSAYED FOR Fe IODOMETRICALLY

Substance	Equivalent of 1 cc. of 0.1*N* $Na_2S_2O_3$, mg.	Official requirement, per cent
U.S.P.		
Ferric ammonium citrate	5.585	Fe = 16.5 to 18.5
Ferric ammonium citrate capsules	5.585	Fe = 15.5 to 19.5*
N.F.		
Beef, iron, and wine	5.585	Fe = 0.75 to 0.975 *W/V*
Ferric ammonium citrate, green	5.585	Fe = 14.5 to 16
Ferric ammonium citrate, green, injection	5.585	Fe = 14.5 to 16*
Ferric cacodylate	5.585	Fe = 11 to 16
Ferric chloride solution	5.585	Fe = 10 to 11
Ferric chloride tincture	5.585	Fe = (n.l.t.) 4.5 *W/V*
Ferric citrochloride tincture	5.585	Fe = (n.l.t.) 4.48
Ferric glycerophosphate	5.585	Fe = (n.l.t.) 17
Ferric hypophosphite	5.585	Fe = (n.l.t.) 21.8
Ferric oxide, red	7.985	Fe_2O_3 = (n.l.t.) 90
Ferric oxide, yellow	7.985	Fe_2O_3 = (n.l.t.) 97.5
Ferric phosphate, soluble	5.585	Fe = 12 to 15
Ferric subsulfate solution	5.585	Fe = 20 to 22
Iron and ammonium acetate solution	5.585	Fe = 0.160 to 0.200 *W/V*
Iron, peptonized	5.585	Fe = 16 to 18
Iron, peptonized, and manganese solution	5.585	Fe = 0.265 to 0.325 *W/V*

* Per cent of labeled amount of ferric ammonium citrate.
(n.l.t.) = not less than.

Exercise 53

Object. Assay of Chlorinated Lime.

Materials Required. About 4 Gm. of chlorinated lime, reagent N.F.
1 Gm. of potassium iodide.
5 cc. of acetic acid.
0.1*N* sodium thiosulfate.
Starch indicator solution.

Procedure. 1. "Transfer to a mortar about 4 Gm. of Chlorinated Lime, accurately weighed in a tared weighing bottle, using 50 cc. of water. Triturate thoroughly, and pour the mixture into a 1000-cc. graduated flask, rinsing the mortar with water to make 1000 cc. Stopper the flask, and allow it to stand for 10 minutes."

The accurately weighed sample of chlorinated lime is triturated with water to disintegrate it to a fine powder.

2. "Shake thoroughly, add to 100 cc. of the mixture 1 Gm. of potassium iodide and 5 cc. of acetic acid, and titrate the liberated iodine with 0.1*N* sodium thiosulfate, starch T.S. being used as the indicator. Each cubic centimeter of 0.1*N* sodium thiosulfate is equivalent to 3.547 mg. of available chlorine (Cl.)"

Chlorinated lime is a product of somewhat indefinite composition which results from the reaction of chlorine with hydrated calcium oxide. It is generally represented by the formula CaOCl(Cl), and part of the chlorine is present as chloride, Cl, and part as hypochlorite, OCl^-. Chlorinated lime in the presence of acids produces chlorine, and "available" or "active" chlorine is usually defined as chlorine which is liberated from a substance by acids.

$$CaOCl(Cl) + 2HOAc \longrightarrow Ca(OAc)_2 + HOCl + HCl$$
$$HOCl + HCl \longrightarrow Cl_2 + H_2O$$

Only a part of the chlorine of chlorinated lime is actually "available" in this sense since the N.F. requires that chlorinated lime "show" not less than 30 per cent of available chlorine although the theoretical chlorine content of CaOCl(Cl) is 55.82 per cent. The chlorine of a sample which is "available" may be determined iodometrically.

$$\underset{2(35.46)}{Cl_2} + 2KI \longrightarrow I_2 + 2KCl$$

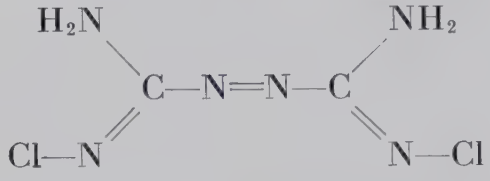
The equivalent weight of chlorine is 35.46 Gm., 1 gram-atomic weight, and each cubic centimeter of 0.1*N* sodium thiosulfate is equivalent to 0.1 × 0.03546 = 0.003546 Gm. or 0.1 meq. of Cl.

Hypochlorous acid which is formed from such compounds as sodium hypochlorite or an *N*-chloroamide, chloramine T, reacts as with potassium iodide in the presence of acetic acid to liberate iodine. In the reaction each Cl^+ is converted into Cl^-, undergoing a change of charge of 2.

$$HOCl + 2KI + HOAc \longrightarrow I_2 + KCl + KOAc + H_2O$$

Since 1 gram-atomic weight of chlorine is equivalent to 1 mole of hypochlorous acid and to 1 mole, 2 equivalents, of iodine, the equivalent weight of the chlorine "available" in the hypochlorite or *N*-chloroamide is 17.73 Gm., ½ gram-atomic weight.

In the stoichiometry involved in the assay of Chloroazodin, each



Cl^+ is converted to Cl^- and 2 equivalents of iodine are consumed by a reaction with the azo linkage. As a result, each mole of Chloroazodin produces 3 moles, 6 equivalents, of iodine and the equivalent weight of Chloroazodin is 30.50 Gm., ⅙ gram-molecular weight.

Questions and Problems

1. By means of half equations show the electronic transfer in the assay of chloroazodin, chlorinated lime, and succinchlorimide.

2. Calculate the equivalent weight, 0.1*N* sodium thiosulfate titer, and the weight of substance that must be in the sample used in the assay procedure, for each of the following, and compare with the corresponding values appearing in the official procedures: (*a*) Halazone tablets ($C_7H_5Cl_2NO_4S$); (*b*) sodium hypochloride solution (NaClO); (*c*) Succinchlorimide tablets ($C_4H_4ClNO_2$).

3. A 4.0050-Gm. sample of chlorinated lime was mixed with enough water to make 1,000 cc. A 100-cc. sample of the mixture was treated with potassium iodide and acetic acid, and titrated with 22.61 cc. of sodium thiosulfate solution. A 20-cc. sample of the sodium thiosulfate solution was found to be equivalent to 0.3680 Gm. of pure iodine. Calculate the "available" chlorine content of the chlorinated lime.

TABLE 30. OFFICIAL SUBSTANCES ASSAYED FOR AVAILABLE CHLORINE IODOMETRICALLY

Substance	Equivalent of 1 cc. of 0.1*N* $Na_2S_2O_3$, mg.	Official requirement, per cent
U.S.P.		
Chloroazodin	3.050	$C_2H_4Cl_2N_6 = 97$ to 102
Chloroazodin solution	0.3050*	$C_2H_4Cl_2N_6 = 0.24$ to $0.28 W/V$
N.F.		
Chloramine T	1.773	Cl, active = 11.5 to 13
Halazone	1.773	Cl, active = 24 to 26.26
Halazone tablets	6.754	$C_7H_5Cl_2NO_4S = 90$ to 135†
Sodium hypochlorite solution	3.723	$NaClO = 4$ to 6
Sodium hypochlorite solution, diluted	3.723	$NaClO = 0.45$ to $0.50 W/V$
Succinchlorimide	1.773	Cl, active = 25 to 27
Succinchlorimide tablets	6.677	$C_4H_4ClNO_2 = 90$ to 130

* 0.01*N* sodium thiosulfate used.

† Per cent of labeled amount.

Exercise 54

Object. Assay of Cupric Sulfate.

Materials Required. 1 Gm. of cupric sulfate.
4 cc. of acetic acid.
3 Gm. of potassium iodide.
0.1*N* sodium thiosulfate.
Starch indicator solution.

Procedure. "Weigh accurately about 1 Gm. of Cupric Sulfate, dissolve it in 50 cc. of water, add 4 cc. of acetic acid and 3 Gm. of potassium iodide, and titrate the liberated iodine with 0.1*N* sodium thiosulfate, using starch T.S. as the indicator. Each cubic centimeter of 0.1*N* sodium thiosulfate is equivalent to 24.97 mg. of $CuSO_4.5H_2O$."

This assay is based on the reaction between cupric sulfate and potassium iodide in which the copper is precipitated as cream-colored cuprous iodide and one atom of iodine is liberated for each cupric ion present:

$$2Cu^{++} + 4I^- \longrightarrow 2Cu^+ + 2I^- + I_2$$

or

$$\underset{2(249.69)}{2CuSO_4.5H_2O} + 4KI \longrightarrow 2CuI + I_2 + 2K_2SO_4 + 10H_2O$$

The equation for the oxidation of potassium iodide with cupric sulfate in the presence of acetic acid shows that the equivalent weight of $CuSO_4.5H_2O$ is 249.69 Gm., 1 gram-molecular weight.

Each cubic centimeter of $0.1N$ sodium thiosulfate is equivalent to $0.1 \times 0.24969 = 0.02497$ Gm. or 0.1 meq. of $CuSO_4.5H_2O$.

The U.S.P. cupric sulfate contains $CuSO_4$ corresponding to not less than 98.5 per cent and not more than 104.5 per cent of $CuSO_4.5H_2O$. Calculate the $CuSO_4.5H_2O$ content of the sample assayed.

Questions and Problems

1. Suggest a method for standardization of $0.1N$ sodium thiosulfate using pure copper, and specify the weight of sample employed so that approximately 35 cc. of the thiosulfate solution will be used in titration.

2. What volume of $0.1056N$ sodium thiosulfate would be required in titration of the iodine equivalent to 0.2 Gm. of $CuSO_4$?

A number of miscellaneous oxidizing agents are assayed iodometrically, and these are listed in Table 31. The method is especially useful in analysis of organoarsenicals since they can be converted quantitatively by oxidative degradation using potassium permanganate and sulfuric acid, to water-soluble inorganic arsenates. The excess permanganate is reduced with hydrogen peroxide and the mixture heated to evaporate any of the excess remaining after all the permanganate has been reduced. By careful treatment of the mixture at this stage first with $0.1N$ permanganate and then with $0.1N$ oxalic acid, a solution containing all the arsenic of the sample in pentavalent form, H_3AsO_4, is obtained. The arsenic acid is then measured iodometrically.

$$H_3AsO_4 + 2KI + 2H^+ \longrightarrow H_3AsO_3 + I_2 + 2K^+ + H_2O$$

Since 1 mole, 2 equivalents, of arsenic acid is formed for each As atom in the organoarsenical molecule, the equivalent weight of Acetarsone, $C_8H_{10}AsNO_5$, is 137.54 Gm., ½ gram-molecular weight, and the equivalent weight of organically bound arsenic is 37.46 Gm., ½ gram-atomic weight.

The ascaridol, organic peroxide, content of chenopodium oil is also assayed iodometrically and this procedure is discussed under the assay of chenopodium oil (page 314).

TABLE 31. MISCELLANEOUS OFFICIAL SUBSTANCES ASSAYED IODOMETRICALLY

Substance	Equivalent of 1 cc. of 0.1*N* $Na_2S_2O_3$ mg.	Official requirement, per cent
U.S.P.		
Cupric sulfate	24.97	$CuSO_4.5H_2O$ = 98.5 to 104.5
N.F.		
Acetarsone	3.746	As = 26.9 to 27.6
Acetarsone tablets	13.75	$C_8H_{10}AsNO_5$ = 92.5 to 107.5*
Chenopodium oil	6.650	$C_{10}H_{16}O_2$ = (n.l.t.) 65
Chenopodium oil capsules	6.650	$C_{10}H_{16}O_2$ = (n.l.t.) 65†
Chromium trioxide	3.334	CrO_3 = (n.l.t.) 98
Glyceryl trinitrate spirit	11.35	$C_3H_5(NO_3)_3$ = 1.0 to 1.1
Neoarsphenamine	3.746	As = (n.l.t.) 19
Sulfarsphenamine	3.746	As = (n.l.t.) 19

* Per cent of labeled amount.
† Per cent of ascaridol in the Chenopodium oil.
(n.l.t.) = not less than.

A residual type of iodometric procedure may be used for substances which react quantitatively with such oxidizing agents as potassium ferricyanide or potassium dichromate. The sample is treated with a carefully measured excess of a standard solution of the oxidizing agent and the excess oxidizing agent is subsequently determined iodometrically. For example, lead acetate reacts with potassium dichromate in the presence of acetic acid as follows:

$$\underset{2(325.30)}{2(C_2H_3O_2)_2Pb} + K_2Cr_2O_7 + H_2O \longrightarrow 2PbCrO_4 + 2C_2H_3O_2K + 2HC_2H_3O_2$$

It should be noted that lead acetate reacts with potassium dichromate, not by reducing it, but by a reaction which goes to completion because of formation of the insoluble lead chromate. The excess potassium dichromate is measured iodometrically. Each cubic centimeter of 0.1*N* potassium dichromate contains 0.1 milliequivalent or $0.1/6 = 0.0166$ millimole of $K_2Cr_2O_7$ and is equivalent to $2 \times 0.0166 = 0.0333$ millimole or $0.0333 \times 0.3253 = 0.01084$ Gm. of $(C_2H_3O_2)_2Pb$.

Table 32. Official Substances Assayed Using Standard $K_2Cr_2O_7$ or $K_3Fe(CN)_6$ in a Residual Iodometric Procedure

Substance	Standard solution	Equivalent of 1 cc., mg.	Official requirement, per cent
U.S.P.			
Benzalkonium chloride	0.05*M* $K_3Fe(CN)_6$	53.60	$[C_6H_5CH_2N(CH_3)_2R]Cl$ = 97 to 103
Benzalkonium chloride solution	0.05*M* $K_3Fe(CN)_6$	53.60	$[C_6H_5CH_2N(CH_3)_2R]Cl$ = 93 to 107*
Quinacrine hydrochloride	0.1*N* $K_2Cr_2O_7$	8.482	$C_{23}H_{30}ClN_3O.2HCl.2H_2O$ = (n.l.t.) 98
Quinacrine hydrochloride tablets	0.1*N* $K_2Cr_2O_7$	8.482	$C_{23}H_{30}ClN_3O.2HCl.2H_2O$ = 95 to 110*
N.F.			
Lead acetate	0.1*N* $K_2Cr_2O_7$	10.84	$C_4H_6O_4Pb$ = 85.3 to 89.6
Proflavine dihydrochloride	0.1*M* $K_3Fe(CN)_6$	62.77	$C_{13}H_{11}N_3$ = 72.0 to 74.5
Proflavine sulfate	0.1*M* $K_3Fe(CN)_6$	62.77	$C_{13}H_{11}N_3$ = 66.0 to 68.5

* Per cent of labeled amount.
(n.l.t.) = not less than.

Benzalkonium chloride reacts quantitatively with standard potassium ferricyanide solution in a mixture buffered with acetate, forming a precipitate of the salt of the quaternary base with ferricyanic acid.

$$\underset{357\ \text{(average m.w.)}}{3[C_6H_5CH_2N(CH_3)_2R]^+Cl^-} + K_3Fe(CN)_6 \longrightarrow [C_6H_5CH_2N(CH_3)_2R]_3Fe(CN)_6 + 3KCl$$

where $R = C_8H_{17}{}^-$ to $C_{18}H_{37}{}^-$
The mixture is filtered and the excess $K_3Fe(CN)_6$ in an aliquot part of the filtrate is determined iodometrically.

$$2K_3Fe(CN)_6 + 2KI + 3ZnSO_4 \longrightarrow I_2 + K_2Zn_3[Fe(CN)_6]_2 + 3K_2SO_4$$

Zinc sulfate is added to prevent oxidation of the ferrocyanide, $Fe(CN)_6^{4-}$, to ferricyanide, $Fe(CN)_6^{3-}$, by the iodine. The potassium zinc ferrocyanide precipitates and the reaction goes to completion. The iodine is titrated in the usual way with 0.1*N* sodium thiosulfate. A 0.05*M* solution of potassium ferricyanide is employed and this is 0.05*N* with respect to the iodometric measurement. Each cubic centimeter of 0.05*M* $K_3Fe(CN)_6$ is equivalent to $3 \times 0.05 = 0.15$ millimole or $0.15 \times 0.357 = 0.05355$ Gm. of $[C_6H_5CH_2N(CH_3)_2R]Cl$.

Exercise 55

Object. Assay of Thyroid.

Materials Required. 1 Gm. of thyroid.

17 Gm. of anhydrous potassium carbonate.

7 cc. of freshly prepared bromine T.S. (prepared by agitating 2 or 3 cc. of bromine with 100 cc. of cold distilled water in a glass-stoppered flask).

5 cc. of a solution of Phenol (1 in 20).

50 cc. of diluted phosphoric acid (1 to 2).

5 cc. of potassium iodide T.S.

0.01*N* sodium thiosulfate.

Starch indicator solution.

Procedure. 1. "Weigh accurately about 1 Gm. of Thyroid in a porcelain crucible, add 7 Gm. of anhydrous potassium carbonate, mix thoroughly, and gently tap the crucible on the desk several times to compact the mixture. Overlay with an additional 10 Gm. of potassium carbonate, and compact the material thoroughly by tapping. Ignite the mixture for 25 minutes at 675° to 700° in a muffle furnace preheated to that temperature."

The active principles of dried thyroid gland are iodine-substituted organic compounds such as thyroxine and diiodotyrosine. When thyroid is subjected to pyrolysis in the presence of potassium carbonate, potassium iodide equivalent to the iodine present in the thyroid sample results. The potassium carbonate employed must be anhydrous; otherwise decrepitation and loss of material would result. The temperature of the muffle furnace and the duration of heating must be carefully regulated since sodium iodide is appreciably volatile at high temperatures and some may be lost on prolonged heating.

2. "Cool, add 20 cc. of water, or more if necessary, heat gently to boiling, and decant through a filter into an Erlenmeyer flask of suitable size. Repeat the

extraction by boiling with 20 cc. of water, then wash the crucible and the char on the filter with hot water until the filtrate measures approximately 200 cc. Add 7 cc. of freshly prepared bromine T.S., then slowly add 40 cc. of dilute phosphoric acid (1 in 2), and boil until starch iodide paper is no longer colored blue by the vapors. During the boiling add water from time to time, as necessary, to maintain a volume of at least 200 cc. Wash down the walls of the flask with water and continue the boiling for 5 min. Cool, add 5 cc. of a solution of phenol (1 in 20), again rinse the walls of the flask and allow to stand for 5 minutes."

After carbonization is complete and the crucible has cooled, the residue is treated with water, which dissolves the KI, carbonates, and other soluble compounds. The filtered solution is treated with bromine T.S. in the presence of phosphoric acid to oxidize the iodide to iodate:

$$I^- + 3Br_2 + 3H_2O \longrightarrow IO_3^- + 6H^+Br^-$$

Most of the excess bromine is removed by heating the solution until the vapors give a negative test with starch iodide paper. The solution must be acidic at this stage; otherwise bromine will be fixed in the form of potassium hypobromite, KBrO. The last traces of bromine are reduced by treatment of the solution with phenol, forming bromophenols such as tribromphenol.

$$C_6H_5OH + 3Br_2 \longrightarrow C_6H_2Br_3OH + 3HBr$$

3. "Add 2 cc. of dilute phosphoric acid (1 in 2) and 5 cc. of potassium iodide T.S., and titrate immediately with 0.01*N* sodium thiosulfate, adding 3 cc. of starch T.S. as the indicator when the end point is neared. Perform a blank test with the same quantities of the same reagents and in the same manner, and make any necessary correction. Each cubic centimeter of 0.01*N* sodium thiosulfate is equivalent to 0.2115 mg. of iodine (I)."

In the presence of a weak acid, IO_3^- oxidizes potassium iodide to an equivalent amount of iodine.

$$IO_3^- + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O$$

Care must be taken to remove the excess bromine in the previous step; otherwise iodide would be oxidized by the bromine and high assay values would result. The iodine formed in the above reaction is titrated with 0.01*N* sodium thiosulfate in the usual way.

Each gram-atomic weight of iodine in thyroid is converted to

1 mole of iodate and subsequently to 3 moles, 6 equivalents, of iodine. Consequently the equivalent weight of the iodine in the thyroid is 21.15 Gm., $^1/_6$ gram-atomic weight, and each cubic centimeter of $0.01N$ sodium thiosulfate is equivalent to 0.01 × 0.02115 = 0.0002115 Gm. of iodine.

The above procedure is also applied with some modifications in he assay of thyroid tablets, U.S.P., and thymol iodide, N.F.

Questions and Problems

1. Why is phosphoric acid used in the oxidation of iodide to iodate and the subsequent oxidation of iodide by the iodate? What reaction would have aken place if a stronger acid, such as hydrochloric, were used?

2. What purpose is served by starch iodide paper and phenol solution in the ssay of thyroid?

3. Calculate the normality of a sodium thiosulfate solution, 48.00 cc. of vhich was required for titration of the iodine formed from KI and 0.3000 Gm. of pure potassium iodate.

4. Consult the N.F. concerning the procedure used for assay of Thymol odide. Outline the steps in the procedure, giving the purpose for each step and each reagent employed.

OXIDATION-REDUCTION METHODS WITH $0.1N$ BROMINE

In the assay of compounds such as aniline, phenol, and resorcinol, bromine is employed as an oxidizing agent in place of iodine since it s reduced by the readily oxidized organic substances in a reaction hat proceeds quantitatively with the production of water-insoluble bromine substitution products such as tribromoaniline, tribromphenol, etc. The standard solution employed does not contain bromine as such but rather an equivalent amount of potassium bromate and excess potassium bromide. Bromine is liberated when he solution is acidified.

Exercise 56

Object. To Prepare and Standardize $0.1N$ Bromine, Koppeschaar's Solution.

Materials Required. 3 Gm. of potassium bromate.
15 Gm. of potassium bromide.
5 cc. of hydrochloric acid.
5 cc. of potassium iodide T.S. (16.5 Gm. of KI in 100 cc.).
$0.1N$ sodium thiosulfate.

Starch indicator solution.

Procedure. "Dissolve 3 Gm. of potassium bromate and 15 Gm. of potassium bromide in sufficient water to make 1000 cc. and determine its exact normality as follows:

"Measure accurately from a burette about 25 cc. of the solution into a 500-cc. iodine flask and dilute with 120 cc. of water. Add 5 cc. of hydrochloric acid, stopper the flask, and shake it gently. Then add 5 cc. of potassium iodide T.S., re-stopper, shake the mixture, allow it to stand for 5 minutes, and titrate the liberated iodine with 0.1*N* sodium thiosulfate, using starch T.S. as the indicator. Calculate the normality and, if desired, adjust exactly to 0.1*N*."

When the solution of potassium bromide and potassium bromate is acidified with hydrochloric acid, bromine is set free:

$$5KBr + KBrO_3 + 6HCl \longrightarrow 6KCl + 3Br_2 + 3H_2O$$

and the liberated bromine oxidizes iodide to an equivalent amount of iodine:

$$2KI + Br_2 \longrightarrow 2KBr + I_2$$

The flask should be tightly stoppered after the addition of the acid to prevent the escape of bromine vapors.

The free iodine is titrated with 0.1*N* sodium thiosulfate to standardize the solution:

$$I_2 + 2Na_2S_2O_3 \longrightarrow 2NaI + Na_2S_4O_6$$

From the volume of sodium thiosulfate solution consumed and its normality, the normality of the bromine solution may be calculated in the usual way.

The equations show that ½ mole of bromine, 1 gram-atomic weight, is 1 equivalent, and ⅙ mole or 27.835 Gm. of potassium bromate, when treated with potassium bromide in presence of hydrochloric acid is required to produce 1 equivalent of bromine. To make 1,000 cc. of 0.1*N* bromine, $0.1 \times 27.835 = 2.7835$ Gm., 0.1 equivalent of $KBrO_3$ is required. However, 3 Gm. of potassium bromate is actually used and the resulting solution should be slightly more concentrated than 0.1*N*. After standardization, the solution may be diluted as necessary, to produce a 0.1*N* solution. Restandardization after diluting is required.

Exercise 57

Object. Assay of Phenol.

Materials Required. 1.5 Gm. of phenol.
30 cc. of 0.1*N* bromine solution.
1 Gm. of potassium iodide.
1 cc. of chloroform.
0.1*N* sodium thiosulfate.
Starch indicator solution.

Procedure. 1. "Dissolve about 1.5 Gm. of Phenol, accurately weighed, n water to make exactly 1000 cc. Transfer an aliquot of the solution, containng from 38 to 41 mg. of Phenol, to an iodine flask, add 30 cc. of 0.1*N* bromine, hen 5 cc. of hydrochloric acid, and immediately insert the stopper. Shake the lask repeatedly during 30 minutes, allow it to stand for 15 minutes, remove the topper just sufficiently to introduce quickly 5 cc. of a solution of potassium odide (1 in 5), being careful that no bromine vapor escapes, and at once stopper he flask."

When the HCl is added, bromine is set free:

$$5KBr + KBrO_3 + 6HCl \longrightarrow 6KCl + 3Br_2 + 3H_2O$$

The bromine reacts with phenol to form a white crystalline preipitate of tribromphenol and hydrobromic acid:

$$\underset{94.11}{C_6H_5OH} + 3Br_2 \longrightarrow C_6H_2Br_3OH + 3HBr$$

The flask should be tightly stoppered to prevent the escape of the olatile bromine and shaken gently during 30 min. to mix the conents thoroughly and allow the oxidation of phenol to tribromphenol o go to completion.

2. "Shake thoroughly, remove the stopper, and rinse it and the neck of the ask with a little water, so that the washings may flow into the flask. Add 1 cc. f chloroform, shake the mixture well, and titrate the liberated iodine with .1*N* sodium thiosulfate, using starch T.S. as the indicator. Perform a blank etermination with the same quantities of the same reagents and in the same anner, and make any necessary correction. Each cubic centimeter of 0.1*N* romine is equivalent to 1.569 mg. of C_6H_6O."

After the addition of potassium iodide, 1 cc. of chloroform is added o dissolve the precipitated tribromphenol which would otherwise nterfere with the clear observation of the end point, particularly in the assay of old, colored phenol. The excess bromine liberates iodine in accordance with the following equation:

$$2KI + Br_2 \longrightarrow 2KBr + I_2$$

The free iodine reacts with sodium thiosulfate:

$$I_2 + Na_2S_2O_3 \longrightarrow 2NaI + Na_2S_4O_6$$

Since 1 mole of phenol reacts with 3 moles, 6 equivalents, of bromine under the conditions of this assay, the equivalent weight of phenol is 15.685 Gm., 1/6 gram-molecular weight. Therefore, each cubic centimeter of $0.1N$ bromine consumed in the reaction with phenol is equivalent to $0.1 \times 0.015685 = 0.001569$ Gm. or 0.1 meq. of phenol (C_6H_6O).

A number of official phenolic substances are assayed in this manner, *e.g.*, hexylresorcinol, phenol, resorcinol, parachlorophenol, and preparations containing these phenols as well as phenolsulfonphthalein and salicylic acid (Table 33).

The reaction of salicylic acid with acidified $0.1N$ bromine is somewhat unusual since decarboxylation and formation of tribromphenol occur.

$$C_7H_6O_3 + 3Br_2 \longrightarrow C_6H_3Br_3O + 3HBr + CO_2$$

The equivalent weight of salicylic acid is 1/6 gram-molecular weight.

Questions and Problems

1. In order to make 1,000 cc. of $0.1N$ bromine, what weight of potassium bromide is theoretically required? How much is actually used?

2. What substance determines the normality of a standard bromine solution?

3. When assayed using $0.1N$ bromine, the products obtained from each of the following substances are given in parentheses. Give equations for the reactions involved and calculate the $0.1N$ bromine titer value for each. (*a*) Hexylresorcinol ($C_{12}H_{16}Br_2O_2$); (*b*) phenolsulfonphthalein injection ($C_{19}H_{10}Br_4O_5S$); (*c*) parachlorophenol ($C_6H_3Br_2ClO$).

4. How much $KBrO_3$ must be present in a solution which, when treated with potassium bromide and hydrochloric acid, will liberate bromine equivalent to 45 cc. of $0.0915N$ sodium thiosulfate? If the volume of the $KBrO_3$ solution were 35 cc., what would be its normality?

5. A 1.4500-Gm. sample of liquefied phenol was dissolved in enough water to make 1,000 cc. A 30-cc. sample of the solution was treated with 30 cc. of oppeschaar's solution and hydrochloric acid. The mixture was treated with otassium iodide and titrated with 7.38 cc. of 0.1*N* sodium thiosulfate. It was lso found that 21 cc. of 0.1*N* sodium thiosulfate was required in titration of he iodine liberated when 20 cc. of the Koppeschaar's solution was treated with otassium iodide and hydrochloric acid. Calculate the per cent of phenol in he original sample.

Exercise 58

Object. Assay of Calcium Hypophosphite.

Materials Required. 0.12 Gm. of calcium hypophosphite.
0.1*N* bromine.
20 cc. of diluted sulfuric acid.
2 Gm. of potassium iodide.
0.1*N* sodium thiosulfate.

Procedure. "Accurately weigh about 120 mg. of Calcium Hypophosphite, ried at 105° for 1 hour, and dissolve it in sufficient water to make 100 cc. ransfer 50 cc. of the solution to a 250-cc. glass-stoppered iodine flask, add 0 cc. of 0.1*N* bromine solution and 20 cc. of diluted sulfuric acid, stopper the ask, place a few cubic centimeters of a saturated potassium iodide solution in he lip around the stopper, shake the flask well, and allow to stand for 3 hours. lace the flask in an ice bath for 5 minutes, then carefully remove the stopper nd allow the potassium iodide solution around the stopper to be drawn into he flask. Add 2 Gm. of potassium iodide dissolved in 10 cc. of recently oiled water, shake the flask, and titrate the liberated iodine with 0.1*N* sodium hiosulfate, using starch T.S. as the indicator. Each cubic centimeter of 0.1*N* romine is equivalent to 2.126 mg. of $Ca(PH_2O_2)_2$."

The hypophosphite ion is oxidized by the acidified 0.1*N* bromine o an equivalent amount of phosphoric acid.

$$\underset{170.07}{a(PH_2O_2)_2} + 4Br_2 + H_2SO_4 + 4H_2O$$

$$\longrightarrow 2H_3PO_4 + 8HBr + CaSO_4$$

One mole of calcium hypophosphite is equivalent to 4 moles, equivalents, of bromine; hence the equivalent weight of calcium ypophosphite is 21.26 Gm., ⅛ gram-molecular weight. Each ubic centimeter of 0.1*N* bromine is equivalent to $0.1 \times 0.02126 =$ .002126 Gm. or 0.1 meq. of $Ca(PH_2O_2)_2$.

Questions and Problems

1. Give equations for all reactions involved in the assay of manganese hypo-hosphite and calculate the equivalent weight and the 0.1*N* bromine titer alue of $Mn(PH_2O_2)_2.H_2O$.

2. What weight of sodium hypophosphite (98.5 per cent $NaPH_2O_2.H_2O$) should be used as sample so that, after employing 50 cc. of 0.0965*N* bromine, 30 cc. of 0.1025*N* sodium thiosulfate would be required?

TABLE 33. OFFICIAL SUBSTANCES ASSAYED BY OXIDATION WITH 0.1*N* BROMINE AND DETERMINATION OF THE EXCESS BROMINE IODOMETRICALLY

Substance	Equivalent of 1 cc., mg.	Official requirement, per cent
U.S.P.		
Hexylresorcinol	4.857	$C_{12}H_{18}O_2$ = (n.l.t.) 98
Hexylresorcinol pills	4.857	$C_{12}H_{18}O_2$ = 91 to 109*
Phenol	1.569	C_6H_6O = (n.l.t.) 98
Phenol, liquefied	1.569	C_6H_6O = (n.l.t.) 88
Phenolsulfonphthalein injection	4.430	$C_{19}H_{14}O_5S$ = 95 to 105*
Resorcinol	1.835	$C_6H_6O_2$ = (n.l.t.) 99.5
Salicylic acid plaster	2.302	$C_7H_6O_3$ = 90 to 110*
N.F.		
Caffeine and sodium salicylate	2.667	$C_7H_5NaO_3$ = 48 to 52
Calcium hypophosphite	2.126	$Ca(PH_2O_2)_2$ = (n.l.t.) 98
Manganese hypophosphite	2.537	$Mn(PH_2O_2)_2.H_2O$ = (n.l.t.) 97
Parachlorophenol	3.214	C_6H_5ClO = (n.l.t.) 99
Phenol ointment	1.569	C_6H_5O = 1.8 to 2.2
Potassium hypophosphite	2.602	KPH_2O_2 = (n.l.t.) 98
Sodium borate solution, compound	1.569	C_6H_6O = 0.25 to 0.31 *W/V*
Sodium hypophosphite	2.650	$NaPH_2O_2.H_2O$ = (n.l.t.) 98

* Per cent of labeled amount.
(n.l.t.) = not less than.

OXIDATION-REDUCTION METHODS WITH POTASSIUM IODATE

Potassium iodate may be used as the oxidizing agent in the assay of a number of substances, such as iodides, arsenites, and other reducing agents. The method depends upon the formation of iodine monochloride in strong hydrochloric acid solution.

Exercise 59

Object. To Prepare 200 cc. of 0.05*M* Potassium Iodate.

Materials Required. About 2.2 Gm. of potassium iodate.

Procedure. Dry 2.2 Gm. of potassium iodate to constant weight at 110°. Dissolve 2.1402 Gm. of the dried salt in sufficient distilled water to make exactly 200 cc. at 25°. The resulting solution should be exactly 0.05 molar.

The potassium iodate is dried to constant weight at 110° to ensure its freedom from moisture. Since potassium iodate is a very stable salt and can be obtained in a very pure condition, standard solutions of this reagent can be prepared by dissolving the calculated weight of the salt in distilled water and diluting to the proper volume.

Standard iodate solutions of known molarity rather than those of known normality are usually employed because the normality varies depending on the nature of the reaction.

The uniform reduction of potassium iodate to iodide, unlike the reduction of potassium bromate to bromide, is generally not feasible in a direct titrimetric procedure and has no application in official assay methods.

$$IO_3^- + 6e + 6H^+ \longrightarrow I^- + 3H_2O \qquad (1)$$

In such a reaction, 1 mole of KIO_3 is 6 equivalents and a $0.05M$ solution would be $0.3N$.

If excess potassium iodate is used, any iodide formed [Eq. (1)] is oxidized to iodine, and the excess iodate and the iodine are determined iodometrically. The reduction of the iodate to iodine may be represented as follows:

$$2IO_3^- + 10e + 12H^+ \longrightarrow I_2 + 6H_2O \qquad (2)$$

In this reaction, 1 mole of iodate is 5 equivalents and a $0.05M$ solution would be $0.25N$. This reaction of iodate is not employed in official assay procedures.

Under certain conditions, the iodide and iodine formed as reduction products of iodate [Eqs. (1) and (2)] are quantitatively converted to I^+. In official procedures, this conversion is accomplished by carrying out the titration in the presence of a rather high concentration of hydrochloric acid. The iodine initially formed by reduction of iodate [Eq. (2)][1] undergoes solvolysis in a polar solvent:

$$I_2 \rightleftharpoons I^+ + I^-$$

The iodine cation forms iodine monochloride when the HCl concentration is sufficiently great,[1] and this is further stablilized by complex ion formation:

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[1] When iodides are assayed using KIO_3, iodine is also formed by oxidation of the iodide.

$$I^+ + HCl \rightleftharpoons ICl + H^+$$
$$ICl + HCl \rightleftharpoons ICl_2^- + H^+$$

or, by summation of these two equations,

$$I^+ + 2HCl \rightleftharpoons ICl_2^- + 2H^+$$

Chloroform or carbon tetrachloride are added to make the end-point observation possible. In the early stages of the titration, iodine is produced and the chloroform layer becomes colored. When all the reducing agent being assayed has been oxidized, the iodate completes the oxidation of iodine and iodide to I^+ and color disappears from the chloroform layer.

If HCN formed from an equivalent amount of KCN in acid solution is used instead of HCl, iodine cyanide, which is much less dissociated than iodine chloride, results. The reaction may be carried out in the presence of not less than $1N$ concentrations of HCl, and starch may be used as indicator. Other substances function in the same way as HCl or HCN in controlling the reduction of IO_3^- to I^+, *e.g.*, Br^- and acetone.

The iodine monochloride procedure is applied in the analysis of a number of official substances. In these procedures, the reduction of KIO_3 may be represented as follows:

$$IO_3^- + 4e + 6H^+ \longrightarrow I^+ + 3H_2O$$

In this reaction, 1 mole of potassium iodate is 4 equivalents and a $0.05M$ solution would be $0.2N$.

Exercise 60

Object. Assay of Potassium Iodide.

Materials Required. 0.5 Gm. potassium iodide.
35 cc. of hydrochloric acid.
5 cc. of chloroform.
$0.05M$ potassium iodate.

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[1] If the solution is saturated with potassium chloride, lower concentrations of hydrochloric acid may be used.

Procedure. "Dry about 500 mg. of Potassium Iodide at 105° for 4 hours, weigh accurately, dissolve it in about 10 cc. of water, and add 35 cc. of hydrochloric acid and 5 cc. of chloroform. Titrate with 0.05*M* potassium iodate until the purple color of iodine disappears from the chloroform. Add the last portions of the iodate solution dropwise, agitating vigorously and continuously. After the chloroform has been decolorized, allow the mixture to stand for 5 minutes. If the chloroform develops a purple color, titrate further with the iodate solution. Each cubic centimeter of 0.05*M* potassium iodate is equivalent to 16.60 mg. of KI."

A number of official iodides are assayed by use of standard potassium iodate, employing the iodine monochloride procedure discussed and illustrated by the assay of potassium iodide. As the end point in the titrations is approached, vigorous shaking is necessary because of the heterogeneous nature of the mixture with iodine and iodate in different phases. The oxidation of potassium iodide by iodate may be represented as follows:

$$\underset{2(166.02)}{2KI} + KIO_3 + 6HCl \longrightarrow 3ICl + 3KCl + 3H_2O$$

As pointed out previously, 1 mole of KIO_3 in this reaction is 4 equivalents; hence a 0.05*M* solution is 0.2*N*. The equivalent weight of potassium iodide is 83.01 Gm., ½ gram-molecular weight. Each cubic centimeter of 0.05*M*, 0.2*N*, potassium iodate is equivalent to $0.2 \times 0.08301 = 0.016602$ Gm. or 0.2 meq. of KI.

Official substances assayed by this procedure (Table 34) include water-soluble inorganic iodides, organic iodine compounds which can be decomposed to iodide (Iodochlorhydroxyquin) and arsenic trioxide in arsenic trioxide tablets.

Potassium bromate is employed as a standard oxidizing agent in the assay of official phenolic substances and certain hypophosphites (Table 33), in the form of standard "bromine" solution. Standard solutions of potassium bromate, without the potassium bromide present, are used for the assay of several official organoarsenicals which can be converted quantitatively into water-soluble trivalent inorganic arsenic compounds. The arsenite obtained from the organoarsenical reduces potassium bromate uniformly to bromide:

$$3AsO_3^{3-} + BrO_3^{-} \longrightarrow 3AsO_4^{3-} + Br^{-}$$

At the end point when all the stronger reducing agent (AsO_3^{3-}) has been oxidized, a slight excess of the potassium bromate solution oxidizes Br^- to Br_2.

$$5Br^- + BrO_3^- + 6H^+ \longrightarrow 3Br_2 + 3H_2O$$

Only a small amount of bromine need be formed in this way to discharge the color of the methyl orange or methyl red added as indicator. The azo dye is oxidized by the bromine to colorless products by a reaction which is somewhat slow; consequently the standard bromate solution must be added very slowly as the end point is approached.

One mole of arsenite, as indicated by the equation, is 2 equivalents. Since 1 gram-atomic weight of arsenic is equivalent to 1 mole, 2 equivalents, of arsenite, the equivalent weight of the arsenic present in the original sample in organic combination is 37.46 Gm., ½ gram-atomic weight. Each cubic centimeter of 0.1*N* potassium bromate is equivalent to $0.1 \times 0.03746 = 0.003746$ Gm. of As. Use is made of a procedure of this type in the assay of carbarsone suppositories for $C_7H_7AsN_2O_4$, dichlorophenarsine hydrochloride for total As, and oxophenarsine hydrochloride for total As.

Questions and Problems

1. Give equations for the reduction of potassium iodate in the presence of phosphoric acid (see Thyroid assay), and for its reduction in the presence of high concentrations of hydrochloric acid.

2. What purpose is served by the hydrochloric acid in the assay of potassium iodide? What other substances may be used in its place?

3. State the differences in composition and application between standard bromine solutions and standard potassium bromate solutions.

4. Forty tablets of red mercuric iodide labeled "1/15-grain tablets of mercuric iodide" weighed 12.4220 Gm. A 10.0000-Gm. sample of the powdered tablets required 14.45 cc. of 0.02*M* KIO_3. Calculate the amount of HgI_2 per tablet. Calculate the per cent of deviation from the labeled amount.

5. The following substances are assayed using 0.05*M* KIO_3 as above described for potassium iodide. Calculate the equivalent weight and the 0.05*M* KIO_3 titer value for each: (*a*) yellow mercurous iodide tablets; (*b*) red mercuric iodide; (*c*) arsenic trioxide tablets.

6. What preliminary treatment is necessary before titration of a sample of iodochlorhydroxyquin with 0.05*M* KIO_3?

7. Calculate the equivalent weight of mercurous iodide and the $0.02M$ KIO_3 titer value for this substance. Note: Hg^+ is oxidized to Hg^{++} by KIO_3.

TABLE 34. OFFICIAL SUBSTANCES ASSAYED BY TITRATION WITH KIO_3

Substance	Molarity of KIO_3	Equivalent of 1 cc., mg.	Official requirement, per cent
U.S.P.			
Iodine solution, strong	0.05	16.60	KI = 9.5 to 10.5 W/V
Iodine tincture	0.05	14.99	NaI = 2.1 to 2.6 W/V
Iodochlorhydroxyquin	0.05	12.69	I = 38 to 41.5
Potassium iodide	0.05	16.60	KI = (n.l.t.) 99
Sodium iodide	0.05	14.99	NaI = (n.l.t.) 99
N.F.			
Arsenic trioxide tablets	0.02	3.956	As_2O_3 = 92.5 to 107.5*
Iodine ampuls	0.05	14.99	NaI = 2.1 to 2.6 W/V
Iodine solution	0.05	14.99	NaI = 2.1 to 2.6 W/V
Iodine tincture, strong	0.05	16.60	KI = 6.8 to 7.5 W/V
Iodochlorhydroxyquin tablets	0.05	30.55	C_9H_5ClINO = 92.5 to 107.5*
Mercuric iodide, red	0.05	22.72	HgI_2 = (n.l.t.) 99
Mercurous iodide, yellow, tablets	0.02	8.734	HgI = 91 to 109*
Potassium iodide solution	0.05	16.60	KI = 97 to 103 W/V
Potassium iodide tablets	0.02	6.641	KI = 94 to 106,† 92.5 to 107.5‡

* Per cent of labeled amount.

† Per cent of labeled amount in tablets containing 300 mg. or more.

‡ Per cent of labeled amount in tablets containing less than 300 mg.

(n.l.t.) = not less than.

Diazotization Assays with Nitrites. The assays of the sulfa drugs and other compounds containing an aryl amino group by diazotization with sodium nitrite are not strictly iodometric methods. They may be included at this point because the end point is determined by the liberation of iodine from iodides.

Exercise 61

Object. To Prepare $0.1M$ Sodium Nitrite.

Materials Required. 7.5 Gm. of sodium nitrite.

Procedure. Dissolve 7.5 Gm. of sodium nitrite in sufficient water to measure 1000 cc. The standardization procedure is identical with that followed in the assay of sulfanilamide, Exercise 62, using a specially purified sulfanilamide as a reference standard.

Since the standardization reaction and procedure are identical with the assay method, the possibility of an end-point error is minimized. It is usually desirable to conduct an exploratory titration before the actual titration. This may be done on a weighed sample, using the outside-indicator after the addition of each cubic centimeter of sodium nitrite solution. In the final standardization titration, sodium nitrite may be added to within about 1 or 2 cc. of the end point found in the exploratory titration, and as the end point is approached, in amounts of 0.1 cc., 0.05 cc., and finally 0.025 cc., testing with the outside-indicator after each addition.

Exercise 62

Object. Assay of Sulfanilamide.

Materials Required. 0.5 Gm. of sulfanilamide.
5 cc. of hydrochloric acid.
30 cc. of 0.1*M* sodium nitrite.
Starch iodide paste T.S.

Procdure. "Weigh accurately about 500 mg. of Sulfanilamide, previously dried at 105° for 2 hours, and transfer it to a beaker or casserole. Add 5 cc. of hydrochloric acid and 50 cc. of water, stir until dissolved, cool to 15°, add about 25 Gm. of crushed ice, and slowly titrate with 0.1*M* sodium nitrite, stirring vigorously, until a blue color is produced immediately when a glass rod dipped into the titrated solution is streaked on a smear of starch iodide paste T.S. When the titration is complete, the end point is reproducible after the mixture has been allowed to stand for 1 minute. Each cubic centimeter of 0.1*M* sodium nitrite is equivalent to 17.22 mg. of $C_6H_8N_2O_2S$."

Nitrous acid, formed when the sodium nitrite solution is introduced into the acid reaction mixture, reacts with the primary amino group of sulfanilamide forming an unstable nitrite which decomposes with formation of a diazonium salt. This product is also unstable and, if the reaction mixture is not kept cold, it will decompose, forming phenol products which are capable of reacting further with nitrous acid. The formation of the diazonium salt may be represented as follows:

$$\underset{172.2}{H_2N{-}SO_2{-}C_6H_4{-}NH_2} + NaNO_2 + 2HCl$$

$$\longrightarrow H_2N{-}SO_2{-}C_6H_4{-}N_2^+Cl^- + NaCl + 2H_2O$$

TABLE 35. OFFICIAL SUBSTANCES ASSAYED BY TITRATION WITH 0.1 *M* NaNO

Substance	Equivalent of 1 cc., mg.	Official requirement, per cent
U.S.P.		
Para-aminobenzoic acid	13.71	$C_7H_7NO_2$ = (n.l.t.) 98.5
Para-aminobenzoic acid tablets	13.71	$C_7H_7NO_2$ = 94 to 106*
Phthalylsulfathiazole	40.34	$C_{17}H_{13}N_3O_5S_2$ = (n.l.t.) 98
Phthalylsulfathiazole tablets	40.34	$C_{17}H_{13}N_3O_5S_2$ = 94 to 106*
Succinylsulfathiazole	35.54	$C_{13}H_{13}N_3O_5S_2$ = (n.l.t.) 99
Succinylsulfathiazole tablets	37.34	$C_{13}H_{13}N_3O_5S_2.H_2O$ = 95 to 105*
Sulfadiazine	25.03	$C_{10}H_{10}N_4O_2S$ = (n.l.t.) 99
Sulfadiazine tablets	25.03	$C_{10}H_{10}N_4O_2S$ = 95 to 105*
Sulfadiazine sodium	27.23	$C_{10}H_9N_4NaO_2S$ = (n.l.t.) 99
Sulfadiazine sodium injection	27.23	$C_{10}H_9N_4NaO_2S$ = 95 to 105*
Sulfadiazine sodium, sterile	27.23	$C_{10}H_9N_4NaO_2S$ = (n.l.t.) 99
Sulfaguanidine	21.42	$C_7H_{10}N_4O_2S$ = (n.l.t.) 99
Sulfaguanidine tablets	23.23	$C_7H_{10}N_4O_2S.H_2O$ = 95 to 105*
Sulfamerazine	26.43	$C_{11}H_{12}N_4O_2S$ = (n.l.t.) 99
Sulfamerazine tablets	26.43	$C_{11}H_{12}N_4O_2S$ = 95 to 105*
Sulfamerazine sodium	28.63	$C_{11}H_{11}N_4NaO_2S$ = (n.l.t.) 99
Sulfamerazine sodium injection	28.63	$C_{11}H_{11}N_4NaO_2S$ = 95 to 105*
Sulfamerazine sodium, sterile	28.63	$C_{11}H_{11}N_4NaO_2S$ = (n.l.t.) 99
Sulfanilamide	17.22	$C_6H_8N_2O_2S$ = (n.l.t.) 99
Sulfanilamide tablets	17.22	$C_6H_8N_2O_2S$ = 95 to 105*
Sulfathiazole	25.53	$C_9H_9N_3O_2S_2$ = (n.l.t.) 99
Sulfathiazole tablets	25.53	$C_9H_9N_3O_2S_2$ = 95 to 105*
Sulfathiazole sodium	27.73	$C_9H_8N_3NaO_2S_2$ = (n.l.t.) 99
Sulfathiazole sodium injection	30.43	$C_9H_8N_3NaO_2S_2.1\frac{1}{2}H_2O$ = 95 to 105*
Sulfathiazole sodium, sterile	27.73	$C_9H_8N_3NaO_2S_2$ = (n.l.t.) 99
N.F.		
Pamaquine naphthoate (for pamaquine base)	31.54	$C_{19}H_{29}N_3O$ = 43 to 45
Procaine borate	23.63	$C_{13}H_{20}N_2O_2$ = 50 to 52
Sulfapyridine	24.93	$C_{11}H_{11}N_3O_2S$ = (n.l.t.) 99
Sulfapyridine sodium, sterile	27.13	$C_{11}H_{10}N_3NaO_2S$ = (n.l.t.) 99
Sulfapyridine tablets	24.93	$C_{11}H_{11}N_3O_2S$ = 95 to 105*

* Per cent of labeled amount.

(n.l.t.) = not less than.

Since 1 mole of sulfanilamide is equivalent to 1 mole of sodium nitrite under the conditions of diazotization, each cubic centimeter of 0.1*M* sodium nitrite, 0.1 millimole, is equivalent to 0.1 × 0.1722 = 0.01722 Gm. or 0.1 millimole of $C_6H_8N_2O_2S$.

At the end point a slight excess of nitrous acid, persisting for at least 1 min., is present. This can be detected by use of starch iodide paste T.S.

$$2I^- + 2HNO_2 + 2H^+ \longrightarrow I_2 + 2NO + 2H_2O$$

A number of official "sulfa" drugs are assayed by this method, including phthalylsulfathiazole and succinylsulfathiazole, which can be diazotized only after a preliminary hydrolysis. The method is applicable also in the assay of para-aminobenzoic acid, procaine borate, and pamaquine base (Table 35).

Chapter 10

GASOMETRIC METHODS

The assay processes that involve the volumetric measurement of gases are of two types, *viz.:*

1. The purity of a gas is determined by absorbing one of the components of a gaseous mixture by means of a suitable absorbing agent and measuring the quantity of gas absorbed by the change in volume.

2. The purity of a given substance is determined by measuring the quantity of gas that it may be made to evolve in a given chemical reaction.

Theory. In assay procedures in which determination of the quantity of a gas is involved, the volume of the gas can be more conveniently and accurately measured than the weight. A simple relationship exists between the volume of a sample of a gas and its weight or mole fraction, and this relationship may be applied in interpretation of experimental data provided corrections are made for temperature and pressure, factors which also determine the volume of a sample of a gas. The relationships between weight or number of molecules, pressure, temperature, and volume of a sample of gas are understandable in the light of the *kinetic-molecular theory.*

The pressure exerted by a gas depends on the number of molecules which strike the walls of the container in a given period of time, on the weight of the molecules and their average velocity. The weight and number of molecules in a given sample of gas are, of course, constant, and the velocity of the molecules depends on temperature. If the temperature is constant, the pressure exerted by a sample of gas depends on the volume, and vice versa. This is true because the number of molecules striking the walls of a small container in a given period of time is greater than it would be if the container were larger, since the molecules need to travel shorter distances in a small container before striking a wall. Therefore, a given weight of gas in which the number of molecules is constant will exert a pressure which is inversely proportional to its volume, provided the temperature is constant. Boyle's law defines the relationship between the volume of a given weight of gas and the pressure exerted by the gas. It may be stated as follows: *the volume of a given weight of gas is inversely proportional to the pressure provided temperature remains constant,*

$$P_1V_1 = P_2V_2 \qquad \text{and} \qquad V_2 = \frac{P_1V_1}{P_2} \tag{1}$$

A given weight of gas will expand or increase in volume when heated at constant pressure, because the average velocity of the molecules and consequently the number of molecules striking the walls of a container are greater at higher temperatures. If the container's volume is fixed, then the gas will exert a greater pressure at the higher temperature. However, if the volume can change, as it can in a gas burette, then the gas expands until it exerts the same pressure in a larger volume which it exerted in a smaller volume before heating. Charles's law, deduced from the kinetic-molecular theory, defines the relationship between the volume of a given weight of gas and the temperature, *viz., the volume of a given weight of gas is directly proportional to the absolute temperature, provided pressure is constant.*

$$\frac{V_1}{T_1} = \frac{V_2}{T_2} \qquad \text{and} \qquad V_2 = \frac{T_2V_1}{T_1} \tag{2}$$

Since the volume of a given weight of gas varies inversely as the pressure (Boyle's law) and directly with the temperature (Charles's law),

$$\frac{P_1V_1}{T_1} = \frac{P_2V_2}{T_2} = K \tag{3}$$

The constant value, K, of the ratio of the product of pressure and volume to absolute temperature of an ideal gas depends on the number of molecules, their mass and velocity. Few gases behave as an ideal gas and, in very precise measurements, corrections must be applied for the volume of the molecules and intermolecular attraction.

The value of K is such that 6.02×10^{23} molecules, 1 mole, of an ideal gas, at 0°C. or 273° absolute and a pressure of 760 mm. of mercury known as *standard conditions*, occupy a volume of 22,400 cc. It follows that 22,400 cc. of any ideal gas, at 0° and 760 mm., contains 6.02×10^{23} molecules or 1 mole, or *equal volumes of gases measured under the same conditions of temperature and pressure contain the the same number of molecules.* This is Avogadro's hypothesis. In order to make use of the 22,400-cc. volume of 1 mole of gas as a conversion factor in calculating mole fractions or weights of a sample of gas from the volume measured in the course of an assay, it is frequently necessary to calculate the volume of the sample at standard conditions from the volume measured under the conditions of the experiment. For example, 40 cc. of NO, measured at 20° and 740 mm. pressure, was obtained in the assay of a 0.2000-Gm. sample of amyl nitrite, $C_5H_{11}NO_2$.

1. What would be the volume of the same sample of NO under standard conditions?

$$V_0 = \frac{P_1 \times V_1 \times T_0}{P_0 \times T_1} \qquad \text{(from Eq. 3)}$$

where V_0 = volume of NO at standard conditions, $P_0 = 760$, $T_0 = 273$, $P_1 = 741$, and $T_1 = 293$. Note that degrees C. + 273 = degrees absolute.

$$V_0 = \frac{40 \times 740 \times 273}{760 \times 293} = 36.3 \text{ cc.}$$

2. What fraction of a mole and what weight of NO were obtained from the amyl nitrite sample?

$$\frac{36.3}{22{,}400} = 0.00162 \text{ mole} \qquad \frac{36.3}{22{,}400} \times 30.01 = 0.0486 \text{ Gm.}$$

3. What was the $C_5H_{11}NO_2$ content of the sample?

The following equation shows the stoichiometric relationship between 1 mole of NO and 1 mole of $C_5H_{11}NO_2$.

$$\underset{2(117.15\text{ Gm.})}{2C_5H_{11}NO_2} + 2KI + H_2SO_4 \longrightarrow 2C_5H_{11}OH + \underset{2(22{,}400\text{ cc.})}{2NO} + I_2 + K_2SO_4$$

Since 36.3 cc. of NO was obtained from a 0.2000-Gm. sample of amyl nitrite, the $C_5H_{11}NO_2$ content of the material may be calculated as follows:

$$\frac{36.3 \times 117.15 \times 100}{22{,}400 \times 0.2} = 94.89 \text{ per cent}$$

Apparatus. The volumetric measurement of gases in the official assay processes is made by the use of a burette of the type illustrated in Fig. 15. The burette should be of 100-cc. capacity and graduated in 0.2-cc. divisions. The burette may be enclosed in a water jacket, as illustrated, to permit the control of temperature. The lower end of the gas burette is attached to an open leveling or equilibrium tube by means of a heavy-walled rubber tube.

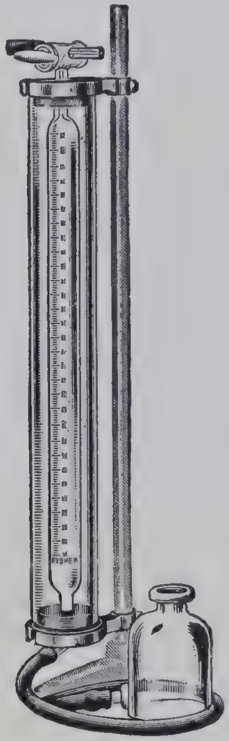

FIG. 15. Gas burette with leveling flask.

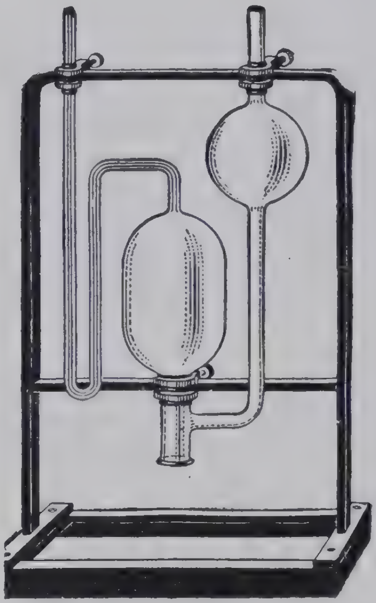

FIG. 16. Simple gas pipette.

The absorption of the gases is accomplished in a gas pipette similar to that shown in Fig. 16. The absorption bulb of the pipette is provided with an opening at the bottom, which can be closed by means of a rubber stopper and through which solid absorbing agents may be introduced.

Exercise 63

Object. Assay of Carbon Dioxide.

Materials Required. Carbon dioxide compressed in a metallic cylinder, which is provided with a reducing valve.

A gas burette with leveling tube.

A gas pipette.

About 2 lb. of mercury.

150 cc. of 50 per cent KOH solution.

Procedure. 1. "Place a sufficient quantity of mercury in a 100-cc. gas burette or nitrometer, provided with a two-way stopcock and a two-way outlet, and properly connected with a balancing tube. Connect one of the outlet tubes of the nitrometer with a gas pipette of suitable capacity. Place in the pipette about 125 cc. of 50 per cent potassium hydroxide solution. Draw the liquid (free from air bubbles) through the capillary opening, connection, and stopcock opening in the nitrometer by reducing the pressure in the nitrometer tube and opening the stopcock controlling the connection with the gas pipette. Then close the stopcock."

The gas burette is filled with mercury by raising the leveling flask containing the mercury until the stopcock opening is filled with the mercury. The stopcock is then closed. The gas pipette is filled with 50 per cent KOH so that the absorption bulb and capillary tube are completely full and the expansion bulb is about one-tenth full and is connected to one of the outlet tubes of the gas burette. Upon opening the stopcock connecting the pipette and the burette and lowering the leveling flask, the KOH solution and any trapped air in the connection are drawn through the stopcock opening. Any air bubbles drawn into the burette may be driven out by opening the stopcock to the other outlet and raising the leveling flask.

2. "Having completely filled the nitrometer, the other stockcock opening, and the other intake tube with mercury, draw into the nitrometer exactly 100 cc. of Carbon Dioxide by reducing the pressure in the tube."

The gas can be introduced directly into the burette from a cylinder of the compressed gas if the latter is provided with a reducing valve that can be carefully controlled. When the gas is obtained in this manner, the connection tubing between the cylinder and the burette should be flushed free of air by allowing a stream of the gas to flow through the tubing for a minute or two. A volume of gas slightly in excess of 100 cc. is then admitted to the burette, and after it has acquired a temperature of 25°, the excess gas is permitted to escape so that a volume of 100 cc. remains.

3. "Close this stopcock. Increase the pressure on the Carbon Dioxide in the nitrometer tube, and open the stopcock controlling the connection with the gas pipette. Force the entire volume of gas into the pipette. Close the stopcock and rock the pipette gently, providing frequent contact of the liquid and the gas. At the end of 5 minutes most of the gas will have been absorbed by the liquid. At this time, to facilitate the absorption of the last portion of the carbon dioxide, draw some of the liquid into the nitrometer tube and force the residual gas back upon the surface of the liquid in the gas pipette. Again rock the pipette until no further diminution in the volume of gas occurs. Draw the residual gas, if any, into the nitrometer tube and measure its volume. Not more than 1 cc. of gas remains."

The CO_2 is absorbed by the KOH forming K_2CO_3, leaving other gases which are not absorbed by KOH as residual gas. If the initial volume of the sample was exactly 100 cc., the decrease in volume is numerically equal to the per cent of CO_2 in the sample.

Official gases which may be assayed by procedures comparable with that used for the assay of carbon dioxide have been listed in Table 36. The methods employed for the assay of cyclopropane, ethylene, and oxygen are quite similar to that used for carbon

TABLE 36. ASSAY OF OFFICIAL GASES

Substance	Method	Official requirement, per cent V/V
U.S.P.		
Carbon dioxide	Absorption, 50 per cent KOH	CO_2 = (n.l.t.) 99
Cyclopropane	Absorption, H_2SO_4	C_3H_6 = (n.l.t.) 99
Ethylene	Absorption, Br T.S.	C_2H_4 = (n.l.t.) 99
Nitrogen	Washing, NH_4Cl—NH_4OH T.S., copper coil	N_2 = (n.l.t.) 99
Nitrous oxide	Condensation	N_2O = (n.l.t.) 95
Oxygen	Absorption, NH_4Cl—NH_4OH T.S., copper coil	O_2 = (n.l.t.) 99

(n.l.t.) = not less than.

dioxide, differing primarily in regard to the reagent used for absorption. Nitrogen is washed free of other gases, especially oxygen, by use of ammonium chloride–ammonium hydroxide T.S. in the presence of a copper wire coil. Nitrous oxide is condensed by use of a specially constructed gas burette and a condensation bulb which is immersed in liquid air (see U.S.P.).

In the U.S.P. assays of amyl nitrite and medicinal zinc peroxide, and ethyl nitrite spirit, N.F., the quantity of gas evolved in a chemical reaction is measured.

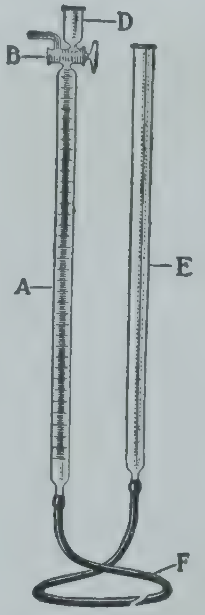

Fig. 17. Lunge nitrometer.

Apparatus. The volumetric measurement of evolved gases may be made by the use of an instrument known as a *nitrometer*. The nitrometer of the type devised by Lunge (Fig. 17) consists of a gas burette *A* of at least 50-cc. capacity, graduated into 0.1 cc., with a special glass stopcock *B* at the top, to which is sealed a glass cup *D*. The gas burette is connected with an open leveling or equilibrium tube *E* by means of a heavy-walled rubber tube *F*.

In the use of the simple Lunge apparatus described above, both tubes of the instrument are partly filled with some liquid in which the gas to be measured is insoluble; generally mercury is employed, but in some cases a saturated salt solution is used. The latter may be used in the measurement of the nitric oxide liberated from ethyl nitrite or amyl nitrite.

When mercury is used, enough of it is poured into the equilibrium tube to fill the burette and leave a slight excess. The stopcock is turned to connect the gas burette with the glass cup, and the equilibrium tube is raised until the mercury level reaches the bottom of the cup to expel all the air from the burette and stopcock bore, and the stopcock is closed. The sample to be assayed, a liquid or a solid in solution, is weighed and poured into the cup from a weighing bottle or weight pipette. Upon opening the stopcock and lowering the equilibrium tube slightly, the sample is drawn into the burette. Care must be exercised to prevent the entrance of air into the passage of the stopcock. The cup is rinsed two or three times with 3-cc. portions of the reagents used to liberate the gas, each being drawn into the burette as described above. With the stopcock closed, the equilibrium tube is lowered sufficiently to cause a slight diminution of the pressure in the burette, and the latter is shaken vigorously for about 5 min. to bring the reagents and sample into intimate contact. When no more gas is evolved on further shaking, the burette is allowed to stand about 30 min. to permit its contents to acquire room temperature. The volume is then measured at atmospheric pressure. Since there is a column of impure reaction mixture above the mercury in the burette, some procedure must be employed to adjust the level of the equilibrium tube so that the weight of the column of mercury in it will just equal the weight of the column of mercury and reaction mixture in the burette. If the reagent mixture has a specific gravity of about 1.3, as it does in the assay for ethyl nitrite, the equilibrium tube is set on the assumption that 1 mm. of mercury will balance about 10 mm. of the reaction mixture. About 5 cc. of dilute sulfuric acid is then poured into the cup, and the stopcock is carefully opened to it. The tendency to draw in or expel gas is noted, and the equilibrium tube is adjusted accordingly. This tedious leveling procedure is obviated in the assay by the use of a saturated solution of sodium chloride in the place of mercury.

Prepare and test a nitrometer as follows: Clean the gas burette and equilibrium tube of the nitrometer thoroughly, connect them by a piece of heavy-walled rubber tubing about 30 in. long, grease the burette stopcock with a thin film of petrolatum, and clamp the assembled instrument securely to a support in a vertical position. Open the stopcock of the burette and pour sufficient saturated solution of sodium chloride into the equilibrium tube to fill the burette and the bore of the stopcock completely when the equilibrium tube is raised. Carefully adjust the level of the equilibrium tube so that the stopcock passage is completely filled with the salt solution without any of it being present in the glass cup and close the stopcock. Lower the equilibrium tube so that the solution level in it corresponds approximately with the 50-cc. graduation on the burette, note the solution level in the burette, and, after the instrument has stood for 20 min., take another reading. If the two readings do not correspond, the stopcock is leaky. If the leakage cannot be corrected by the use of stopcock grease, another instrument should be employed.

Exercise 64

Object. Assay of Ethyl Nitrite Spirit.

Materials Required. 1 Lunge nitrometer.
1 10-cc. delivery pipette.
1 100-cc. volumetric flask.
40 cc. of ethyl nitrite spirit.
0.5 Gm. of potassium bicarbonate.
60 cc. of alcohol.
10 cc. of potassium iodide T.S.
5 cc. of diluted sulfuric acid.

Procedure. 1. "Transfer about 40 cc. of Ethyl Nitrite Spirit which has been previously shaken with 500 mg. of powdered potassium bicarbonate, to a tared, 100-cc. volumetric flask, and weigh accurately. Add sufficient alcohol to bring the volume to exactly 100 cc., and mix thoroughly."

In the presence of water, ethyl nitrite is partially hydrolyzed with the formation of ethyl alcohol and nitrous acid:

$$C_2H_5NO_2 + H_2O \longrightarrow C_2H_5OH + HNO_2$$

Since the nitrous acid, if present, would liberate oxide in the assay, it is removed by shaking the spirit with powdered potassium bicarbonate:

$$HNO_2 + KHCO_3 \longrightarrow KNO_2 + H_2O + CO_2$$

The potassium nitrite formed is practically insoluble in the spirit. The sample is diluted with alcohol to 100 cc. after weighing, so that an aliquot portion of the solution representing a definite weight of the spirit may be measured into the nitrometer, because it is difficult to weigh and transfer a sample of the spirit directly without the loss of some ethyl nitrite.

2. "Introduce into the funnel top of a nitrometer which has previously been filled with a saturated solution of sodium chloride, exactly 10 cc. of the alcohol solution, and after drawing this into the measuring tube of the nitrometer without the admission of air, follow it successively with 5 cc. of alcohol as a rinse, then with 10 cc. of potassium iodide T.S., and afterward with 5 cc. of

diluted sulfuric acid, introducing each reagent separately into the measuring tube. When the volume of gas has become constant (within 30 to 60 minutes), note the volume of gas collected and also the temperature at the nitrometer as well as the barometric pressure."

Adjust the nitrometer prepared for use in the preceding exercise so that the level of the salt solution in the equilibrium tube is about even with the 15-cc. graduation mark on the burette, the latter as well as the stopcock passage being filled with the salt solution. Fill a 10-cc. pipette with the diluted spirit exactly to the graduation mark and transfer the liquid into the nitrometer cup. Open the stopcock at once to allow the solution to be drawn into the burette, using care to prevent the entrance of air at the same time. Rinse the cup at once with two 5-cc. portions of potassium iodide solution (1.65 Gm. of KI in 10 cc. of H_2O), drawing each portion into the burette by lowering the equilibrium tube, if necessary, and then place about 6 cc. of diluted sulfuric acid in the cup and draw about 5 cc. of it into the burette in the same manner. The excess acid remaining in the cup serves to prevent the escape of the nitric oxide gas, which begins to form as soon as the first of the acid is introduced, and it prevents the entrance of air. After the stopcock has been tightly closed and the equilibrium tube well clamped to the support, remove the burette from the clamp, incline it at an angle of about 45 deg., and shake it vigorously for about 5 min. to mix the reagents thoroughly with the solution of ethyl nitrite.

Potassium iodide, in the presence of sulfuric acid, reduces ethyl nitrite (C_2H_5ONO) to an equivalent amount of nitric oxide (NO) as follows:

$$\underset{2(75.07\text{ Gm.})}{2C_2H_5ONO} + 2KI + H_2SO_4 \longrightarrow 2C_2H_5OH + \underset{2(22,400\text{ cc.})}{2NO} + I_2 + K_2SO_4$$

The nitric oxide gas formed, being insoluble in saturated salt solution, rises to the top of the burette, and after it has acquired room temperature, as shown by the constancy of its volume when two successive readings taken at 10-min. intervals agree, the volume of gas liberated is recorded. When the final reading of the volume of gas is taken, the liquids in the burette and equilibrium tube should be on the same level.

3. "Multiply this volume in cubic centimeters by 0.307, and divide the product by one-tenth of the weight of the Ethyl Nitrite Spirit taken. At 25° and 760 mm. pressure, the quotient represents the percentage of ethyl nitrite in the liquid. The temperature correction is $^1/_{273}$ of the total percentage just found for each degree of temperature, added if the temperature is below, subtracted if above, 25°. The barometric correction is $^1/_{760}$ of the total percentage, after correction for temperature, for each mm., added if above, subtracted if below, 760 mm."

If the nitric oxide, collected at 25° and 760 mm. pressure, has a volume V_{25}, the volume at 0° would be

$$V_0 = V_{25} \times \frac{273}{298}$$

Since 75.07 Gm. or 1 mole of C_2H_5ONO would have produced 22,400 cc. of NO at 0° and 760 mm. pressure, the amount of C_2H_5ONO which produced the above corrected volume of NO must have been

$$\frac{V_{25} \times {}^{273}/_{298}}{22,400} \times 75.07$$

or

$$V_{25} \times \frac{273}{298} \times \frac{75.07}{22,400}$$

or

$$V_{25} \times 0.00307 \text{ (Gm. of } C_2H_5ONO)$$

In other words, each cubic centimeter of nitric oxide, collected at 25° and 760 mm., is equivalent to 0.00307 Gm. of C_2H_5ONO. This amount of C_2H_5ONO was in a $^1/_{10}$ aliquot part of the sample. Therefore, in 100 Gm. of the material, there must have been the following per cent of C_2H_5ONO:

$$\frac{V_{25} \times 0.00307}{\text{Wt. of sample}/10} \times 100 = \frac{V_{25} \times 0.307}{\text{wt. of sample}/10} = \text{per cent } C_2H_5ONO$$

The N.F. directs that the volume observed at 25° and 760 mm. be multiplied by 0.307 and the product divided by one-tenth the weight of the sample of ethyl nitrite spirit. As indicated above, the result is the number of grams of C_2H_5ONO in 100 Gm. of ethyl nitrite spirit, *i.e.*, per cent *W/W*.

The corrections for the deviation of temperature from 25° and for the deviation of pressure from 760 mm. are made in accordance with the gas laws, as directed in the procedure. For example, a 40-Gm. sample of ethyl nitrite spirit yielded 50 cc. of NO, measured at 21° and 740 mm. If the NO had been measured at normal conditions, the C_2H_5ONO content would have been

$$\frac{50 \times 0.307}{4} = 3.84 \text{ per cent}$$

After correcting for the deviation of temperature,

$$3.84 + (4/273 \times 3.84) = 3.896$$

and for the deviation in pressure,

$$3.896 - (20/760 \times 3.84) = 3.612$$

the C_2H_5ONO content of the sample is calculated as 3.61 per cent.

Comment. When a gas is collected over a liquid, the pressure of the gaseous mixture is equal to the pressure of the gas plus the pressure of the vapors of the liquid in accordance with Dalton's law of partial pressures. In the above experiment, no correction was made for vapor pressure of the liquid over which the nitric oxide was collected. The liquid consisted of a saturated solution of sodium chloride to which had been added, in the course of the experiment, a small amount of water contained in potassium iodide T.S. and diluted sulfuric acid and a small amount of alcohol. Since dissolved salts lower the vapor pressure of the solvent, and since the liquid used in this experiment was practically saturated with sodium chloride, any error arising from the vapor pressure of the liquid is very small and probably compensated by the slight solubility of nitric oxide in the liquid.

Questions and Problems

1. A mixture of the following three samples of gas was prepared: 50 cc. of carbon dioxide, measured at 21°, 750 mm.; 25 cc. of oxygen, measured at 28°, 740 mm.; and 30 cc. of nitrogen, measured at 26°, 765 mm. (*a*) What is the volume of the mixture at normal conditions? (*b*) What is the volume at standard conditions after shaking the mixture with 50 per cent potassium hydroxide?

2. If 2.5 Gm. of oxygen is collected over water at 25° and 760 mm. under conditions such that the gas is saturated with water vapor, what is the volume of the mixture of oxygen and water vapor? What would be the volume of the oxygen if no water vapor were present?

3. By what factor must the volume of nitric oxide produced by a sample of amyl nitrite, $C_5H_{11}NO_2$, and collected at 25° and 760 mm., be multiplied so that the ratio of this product to the weight of the sample of amyl nitrite employed will be the percentage concentration of $C_5H_{11}NO_2$ in the sample? Compare the calculated value with that given in the U.S.P. assay procedure.

4. What chemical reactions take place in the assay of cyclopropane, ethylene, and medicinal zinc peroxide?

5. A $^1/_{10}$ aliquot part of a 42-Gm. sample of ethyl nitrite spirit evolved 48 cc. of nitric oxide measured at 28° and 750 mm. pressure. Calculate the per cent C_2H_5ONO of the sample.

PART 2

SPECIAL METHODS USED IN OFFICIAL PHARMACEUTICAL ANALYSES

Quantitative analyses of crude drugs and of the products derived from them are made to establish purity or to determine the amount of therapeutically active constituents present for the purpose of standardization. The special methods employed in the analyses of this type may be classified as follows:

Chemical methods, such as those employed in the determination of ash, moisture, crude fiber, extractive obtained with different solvents, the estimation of alkaloidal content, etc.

Biological methods, or those in which the effects of drugs upon animals or animal tissues are measured and which are employed in the absence of satisfactory chemical methods for standardization. Official standards for drugs or preparations include: biological assays or tests for aconite, vitamins A and D, digitalis, epinephrine, histidine, insulin, liver, stomach, ouabain, parathyroid, pituitary, and strophanthus. Tests are required for the toxicity and purity of dichlorphenarsine hydrochloride, neoarsphenamine, oxophenarsine hydrochloride, and suramin sodium and for serological preparations such as serums, vaccines, toxins, and antitoxins. Biological methods require special apparatus for their performance and a technique that is not chemical in nature; hence a discussion of them does not fall within the scope of this text.

Chapter 11

ASH AND MOISTURE DETERMINATIONS

Ash Content. The ash content of a crude drug is generally taken to be the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also include inorganic matter added for the purposes of adulteration. There is a considerable difference in the ash content of different drugs, but the difference varies within narrow limits in the case of the same individual; hence, an ash determination furnishes a basis of judging the identity and cleanliness of a drug and gives information relative to its adulteration with inorganic matter. Ash standards have been established for a number of the official drugs; usually these standards set a maximum limit on the total ash or on the acid-insoluble ash permitted. The *total ash* is the residue remaining after incineration, the *acid-insoluble ash* is that part of the total ash which is insoluble in diluted hydrochloric acid. The Pharmacopoeia specifies that all vegetable drugs shall be separated by mechanical means in so far as possible from lumps of dirt or other foreign inorganic matter before they are ground or powdered. When no ash standard is fixed for a given drug by the Pharmacopoeia or the National Formulary, the amount of foreign inorganic matter estimated as acid-insoluble ash must not exceed 2 per cent of the weight of the drug.

The ash or residue yielded by an organic chemical compound is, as a rule, a measure of the amount of inorganic matter present as impurity. In most cases, the inorganic matter is present in small amounts, which are difficult to remove in the purification process and which are not objectionable if only traces are present.

The careful control of temperature is the most important analytical factor to regulate in making ash determinations. All determinations should be made in such a manner as to duplicate in so far as possible the conditions under which standards are established. When an electric furnace is used for ignitions, the following temperature equivalents, which are only approximate, will be found of value: Very dull red heat = 500 to 550°; dull red heat = 550 to 700°; bright red heat = 800 to 1000°; yellow red heat = 1000 to 1200°; and white heat = 1200 to 1600°. Practically all these temperatures are within the limits of the ordinary Bunsen burner, depending on the construction of the burner and the nature of the gas burned. When it is considered that calcium carbonate is converted to calcium oxide at about 825°, that calcium phosphate is converted to calcium pyrophosphate at about 1550°, that lithium carbonate forms lithium oxide at about 600°, and that potassium chloride sublimes at about 1500°, it becomes apparent that ignitions should be carried out at low temperatures if possible.

Exercise 65

Object. To Determine the Total and Acid-insoluble Ash Content of Digitalis Leaf.

Materials Required. 2 to 4 Gm. of the ground drug (see sampling, page 15).

2 ashless filter papers.

Procedure. 1. "Accurately weigh a quantity of the ground drug, representing from 2 to 4 Gm. of the air-dried material, in a tared crucible and incinerate at a low temperature, not to exceed very dull redness, until free from carbon, and determine the weight of the ash."

The crucible in which the drug is to be ignited should be heated to dull redness until of constant weight, cooled in a desiccator, and weighed. This procedure drives off the moisture and the absorbed gases that are present on the surface of the crucible. The drug is incinerated at a temperature not to exceed a dull redness. Very often, the ash consists largely of calcium carbonate formed from the calcium oxalate contained in the drug. When the incineration is carried out at a dull red heat, the calcium oxalate is converted into carbonate, but when the incineration is carried out at a bright red heat, varying amounts of the carbonate are converted into the oxide with consequent variable results. If the ash contains alkali chlorides, which it frequently does, there may be a loss of some chloride by volatilization when a high ignition temperature is used.

2. "If a carbon-free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the insoluble residue on an ashless filter paper, incinerate the residue and filter paper until the ash is white or nearly so, then add the filtrate, evaporate it to dryness and heat the whole to a low redness. If a carbon-free ash cannot be obtained in this way, cool the crucible, add 15 cc. of alcohol, break up the ash with a glass rod, burn off the alcohol, and again heat the whole to a low redness. Finally determine the weight of the ash. Calculate the percentage of total ash from the weight of the drug taken."

When the ignition residue is not heated above dull redness, it is frequently very difficult to burn off all the carbon, some of which may become surrounded by fused inorganic matter, thereby keeping it from coming in contact with the oxygen of the air. In such cases, the residue is treated with hot distilled water to dissolve most of the inorganic matter, and the insoluble portion is collected on an ashless filter and incinerated. The filtrate is then added to this residue, evaporated, and the whole is ignited to burn off any colloidal carbon that may have passed into the filtrate. Occasionally, it may be necessary to treat the residual mass with alcohol to disintegrate the fused lump, especially in the ignition of resinous drugs.

The weight of the crucible and ash minus the weight of the crucible gives the weight of total ash obtained from the sample of drug taken. This total ash, which usually contains carbonates, phosphates, sulfates, chlorides, oxides, etc., of calcium, magnesium, potassium, sodium, aluminum, iron, and other metallic elements, does not necessarily represent all the inorganic constituents of the drug, since ammonium salts, some alkali iodides and nitrates, etc., are volatilized or converted into carbonates, etc.

3. "Boil the ash obtained with 25 cc. of diluted hydrochloric acid for five minutes, collect the insoluble matter on a tared Gooch crucible or on an ashless filter, wash with hot water, ignite, and weigh. Determine the per cent of acid-insoluble ash calculated from the weight of drug taken."

The diluted hydrochloric acid dissolves the calcium carbonate, alkali chlorides, etc., leaving an acid-insoluble residue that consists almost entirely of silica derived from the soil, adhering to the drug.

Questions and Problems

1. From what different sources may the ash of a drug be derived?
2. When no specific ash limit is set by the official standards, what per cent of acid-insoluble ash is the drug permitted to contain?
3. Why should the incineration residue not be heated above a dull redness?
4. Name the important inorganic plant constituents that will not be found in the ash.
5. What does the quantity of acid-insoluble ash in a drug indicate?

The ash content of chemicals is determined by ignition to dull redness in the same manner as in the determination of the ash content of a crude drug.

It is economically impracticable to make ash determinations on large samples of expensive chemicals. However, it is necessary to control the amount of inorganic matter that may be contained in such substances as alkaloidal salts, especially when the substance is intended for hypodermic administration. For this reason, the official standards usually require that small amounts of expensive substances be used and that the ash yielded be negligible. The term *negligible* is defined as a quantity not exceeding 0.0005 Gm. A number of official substances, with the quantity of the substance in grams in parentheses, required to yield a negligible amount of residue are: **U.S.P.** (0.2) apomorphine hydrochloride, (0.2) bishydroxycoumarin, (0.5) cocaine, (0.5) cocaine hydrochloride, (0.5) codeine sulfate, (0.1) colchicine, (0.1) digitoxin, (0.1) digoxin, (0.2) dihydromorphinone hydrochloride, (0.2) emetine hydrochloride, (0.1) epinephrine, (0.1) estradiol, (0.1) estradiol benzoate, (0.1) estrone, (0.2) ethylmorphine hydrochloride, (0.1) folic acid, (0.2) homatropine hydrobromide, (0.25) homatropine methylbromide, (0.1) lanatoside C, (0.1) ouabain, (0.1) physostigmine salicylate, (0.2) picrotoxin, (0.2) pilocarpine hydrochloride, (0.2) pilocarpine nitrate, (0.5) morphine sulfate, (0.2) quinacrine hydrochloride, (0.1) scopolamine hydrobromide, (0.1) triethanolamine; **N.F.** (0.5) anthralin, (10 cc.) benzyl alcohol, (0.5) codeine, (0.5) dibucaine hydrochloride, (1.0) histidine monohydrochloride, (0.1) morphine hydrochloride, (1.0) salicylanilide, (0.2) succinchlorimide.

The ash limits of most of the official vegetable drugs and chemicals are indicated in Table 37. The per cent of ash given is the maximum permitted for each substance.

Residue limits are given in the official standards for a number of solvents and preparations. The residue is determined by evaporation and drying to constant weight at a specified temperature or by evaporation and ignition of the residue as indicated in Table 38.

Moisture Content. The moisture content of crude drugs is somewhat variable, since most vegetable drugs are more or less hygroscopic. Some drugs take up as much as 5 per cent of their air-dried weight when stored in a humid atmosphere. Generally, assays are made to determine the proportion of active constituents contained in a drug in the condition in which it is purchased or used. In accurate scientific work, however, and in those cases where the drug is to be sold with a guaranteed assay, the per cent of active constituent must be calculated on the basis of the moisture-free drug.

The method most commonly followed in determining the moisture content of a drug is to heat it at 100° in an oven until the weight becomes constant. Since, however, most vegetable drugs contain variable amounts of volatile substances other than water, such as essential oils, ethers, and esters, amines and alkaloids in some instances, this method is not generally applicable except for rough estimations. When the drug contains volatile matter other than water, the U.S.P. and the N.F. directions require that the volatile ether-soluble extractive shall be determined and the weight of the latter subtracted from the weight lost by the drug upon drying, the difference to be taken as the moisture content of the drug. Even this procedure is not free from error. Since water is slightly miscible with ether, some of the moisture contained in the drug is extracted by the anhydrous ether and determined as volatile ether-soluble extractive. A better

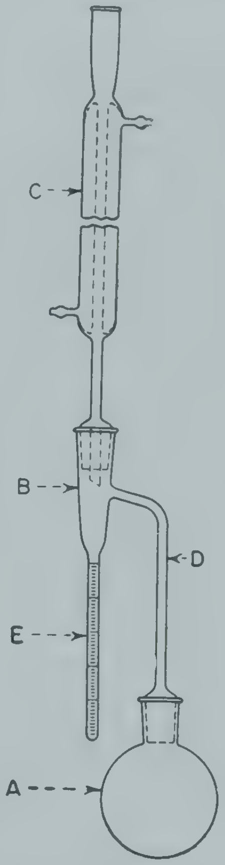

Fig. 18. Toluene moisture tube.

TABLE 37. OFFICIAL SUBSTANCES WITH ASH LIMITS

Substance	Total ash, per cent	Acid-insoluble ash, per cent
U.S.P.		
Acacia	4.0	0.5
Acetanilid	0.05	
Acetophenetidin	0.05	
Acetylsalicylic acid	0.05	
Agar	6.5	0.5
Aloe	4.0	
Aloin	0.6	
Aminophylline	0.15	
Aminopyrine	0.15	
Ammonium carbonate	0.05	
Ammonium chloride	0.1	
Amphetamine sulfate	0.2	
Ascorbic acid	0.1	
Aspidium		3.0
Atropine	0.1	
Atropine sulfate	0.2	
Barbital	0.1	
Belladonna leaf		3.0
Benzalkonium chloride	0.2	
Benzoic acid		0.05
Benzoin, Sumatra		1.0
Benzoin, Siam		0.5
Butacaine sulfate	0.2	
Cacao	8.0	0.4
Caffeine	0.1	
Caraway		1.5
Carbachol	0.1	
Cardamon seed		4.0
Cellulose, oxidized	0.15	
Cherry juice*		
Chloral hydrate	0.1	
Chloroazodin	0.1	
Chlorobutanol	0.1	
Chloroguanide hydrochloride	0.1	
Chlorophenothane	0.05	
Cholesterol	0.1	
Chrysarobin	0.3	
Citric acid	0.05	
Congo red	20.0 to 24.0	
Congo red injection	20.0 to 24.0	
Cotton, purified	0.2	
U.S.P.		
Dextrose	0.1	
Diethylstilbestrol	0.05	
Digitalis		5.0
Digitalis, powdered		5.0
Diiodohydroxyquinoline	0.5	
Diphenhydramine hydrochloride	0.1	
Ephedrine	0.1	
Ephedrine hydrochloride	0.1	
Ephedrine sulfate	0.1	
Ethyl aminobenzoate	0.1	
Eucatropine hydrochloride	0.1	
Gauze, absorbent	0.15	
Gauze, bandage	0.15	
Gelatin	2.0	
Glucose, liquid	0.5	
Glycerin	0.01	
Glycyrrhiza		2.5
Glycyrrhiza extract	8.0	
Heparin sodium	41.0	
Hexylresorcinol	0.1	
Hydriodic acid, diluted	1.8	
Hydrochloric acid	0.01	
Hydrochloric acid, diluted	0.01	
Insulin injection**		
Iodized oil	0.1	
Iodoalphionic acid	0.3	
Iodochlorhydroxyquin	0.5	
Iodopyracet injection	0.1	
Lactic acid	0.1	
Lactose	0.1	
Menadione	0.1	
Meperidine hydrochloride	0.1	
Meralluride	0.1	
Mercuric oxide, yellow	0.2	
Mercury, ammoniated	0.2	
Mercury bichloride	0.1	
Methacholine chloride	0.1	

* 35 to 55 mg. per 10 cc.

** 1.0 mg. per 1,000 Insulin Units.

TABLE 37. OFFICIAL SUBSTANCES WITH ASH LIMITS. (*Continued*)

Substance	Total ash, per cent	Acid-insoluble ash, per cent
U.S.P.		
Methamphetamine hydrochloride	0.1	
Methenamine	0.05	
Methylparaben	0.1	
Naphazoline hydrochloride	0.2	
Neocinchophen	0.1	
Neostigmine bromide	0.15	
Neostigmine methylsulfate	0.1	
Nicotinamide	0.1	
Nicotinic acid	0.1	
Nikethamide	0.05	
Oleic acid	0.1	
Papaverine hydrochloride	0.5	
Para-aminobenzoic acid	0.1	
Petrolatum	0.05	
Phenobarbital	0.15	
Phenolphthalein	0.1	
Phenolsulfonphthalein	0.2	
Phenylephrine hydrochloride	0.2	
Phthalylsulfathiazole	0.1	
Piperocaine hydrochloride	0.15	
Polyethylene glycol 4000	0.25	
Polysorbate 80	0.15	
Procaine hydrochloride	0.15	
Propylene glycol	0.07	
Propylparaben	0.05	
Propylthiouracil	0.1	
Pyroxylin	0.3	
Quinidine sulfate	0.1	
Quinine dihydrochloride	0.15	
Quinine hydrochloride	0.15	
Quinine sulfate	0.05	
Raspberry juice*		
Resorcinol	0.05	
Riboflavin	0.3	
U.S.P.		
Saccharin	0.2	
Salicylic acid	0.05	
Sarsaparilla (Mexican)		4.0
Sponge, absorbable, gelatin	2.0	
Starch	0.5	
Succinylsulfathiazole	0.1	
Sucrose	0.05	
Sulfadiazine	0.1	
Sulfaguanide	0.1	
Sulfamerazine	0.1	
Sulfanilamide	0.1	
Sulfathiazole	0.1	
Sulfur, precipitated	0.3	
Tar, coal	2.0	
Tartaric acid	0.05	
Tetracaine hydrochloride	0.1	
Theophylline	0.15	
Thiamine hydrochloride	0.2	
Tribromoethanol	0.1	
Trichloroacetic acid	0.05	
Tripelennamine hydrochloride	0.1	
Tubocurarine chloride	0.25	
Tyrothricin	3.5	
Urea	0.1	
Urethan	0.1	
Vanillin	0.05	
Wool fat	0.1	
Yeast, dried	8.0	
N.F.		
Acetarsone	0.2	
Acriflavine	3.5	
Acriflavine hydrochloride	1.0	
Althea		1.0
Aminoacetic acid	0.1	
Ammonium acetate solution	0.015	
Ammonium bromide	0.05	
Ammonium iodide	0.5	

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* 25 to 45 mg. per 10 cc.

TABLE 37. OFFICIAL SUBSTANCES WITH ASH LIMITS. *(Continued)*

Substance	Total ash, per cent	Acid-insoluble ash, per cent
N.F.		
Ammonium salicylate	0.05	
Ammonium valerate, acid	0.05	
Amobarbital	0.1	
Anise		1.5
Antipyrine	0.15	
Aralia		2.0
Areca	2.5	
Arecoline hydrobromide	0.5	
Arnica		2.0
Aromatic solution, alkaline	2.23 to 2.73	
Arsenic trioxide	0.1	
Asafetida		15.0
Beef extract*		
Belladonna root		4.0
Betanaphthol	0.1	
Brucine sulfate	0.1	
Buchu		1.0
Butyl aminobenzoate	0.15	
Caffeine, citrated	0.1	
Camphor, monobromated	0.05	
Capsicum		1.25
Caramel	8.0	
Carbromal	0.1	
Carmine	12.0	
Carmine solution	0.8	
Charcoal, activated	4.0	
Chlorothymol	0.05	
Chondrus		2.0
Chondrus extract	29.0	1.0
Cinchona		2.0
Cinchonidine sulfate	0.1	
Cinchonine sulfate	0.1	
Cincophen	0.25	
Cinnamon, Ceylon		2.0
Clove		0.75
Colchicum corn		0.5
Colchicum seed		1.0
Colocynth		4.0
Coriander		1.5
N.F.		
Creosote carbonate	0.1	
Cubeb		2.0
Dehydrocholic acid	0.3 (oral use) 0.1 (parenteral use)	
Dioctyl sodium sulfosuccinate	16.0 to 17.0	
Elm		1.0
Eriodictyon		2.0
Fennel		1.5
Frangula		2.0
Gamboge		1.0
Gelsemium		2.0
Glutamic acid hydrochloride	0.1	
Glyceryl monostearate	0.1	
Grindelia		2.0
Guaiac		2.0
Guiacol	0.1	
Hamamelis leaf		2.0
Honey	0.3	
Hydrastis		3.0
Hyoscyamus		12.0
Ichthammol	0.5	
Iodoform	0.2	
Ipomea		3.0
Ipomea resin	0.5	
Jalap		0.5
Juniper		2.0
Larkspur		2.0
Leptandra	6.0	
Linseed		1.0
Liver, desiccated	7.0	
Lobelia		5.0
Lycopodium		0.75
Mandelic acid	0.1	
Mastic		0.25
Mercuric iodide, red	0.2	
Mercuric oxide, red	0.2	
Mercuric salicylate	0.1	
Mercuric succinimide	0.1	
Mercurous chloride, mild	0.1	

* 30 per cent of total solids.

TABLE 37. OFFICIAL SUBSTANCES WITH ASH LIMITS. *(Continued)*

Substance	Total ash, per cent	Acid-insoluble ash, per cent
N.F.		
Mercurous iodide, yellow	0.1	
Mercury	0.01	
Methylcellulose	0.5	
Methylene blue	1.2	
Methylrosaniline chloride	1.5	
Myristica	0.5	
Myrrh		5.0
Nitric acid	0.0066	
Nitromersol	0.1	
Nutgall		2.0
Nux vomica		1.0
Orris		1.0
Pamaquine naphthoate	1.0	
Pectin	4.0	0.4
Phenacaine hydrochloride	0.15	
Phenothiazine	0.2	
Phenylmercuric chloride	0.1	
Phenylmercuric nitrate	0.1	
Phenyl salicylate	0.1	
Plantago seed	4.0	1.0
Podophyllum		2.0
Podophyllum resin	1.5	
Poplar bud		1.0
Proflavine dihydrochloride	0.25	
Proflavine sulfate	1.0	
Pyrethrum		2.0
Pyrogallol	0.1	
Quassia		0.5
Quillaja		2.0
Quinine	0.1	
Quinine and urea hydrochloride	0.15	
Quinine bisulfate	0.05	
Quinine hydrobromide	0.05	
N.F.		
Racephedrine hydrochloride	0.1	
Resorcinol monoacetate	0.1	
Rosin	0.1	
Rutin	0.5	
Salicin	0.05	
Sanguinaria		2.0
Santonin	0.1	
Sassafras		5.0
Scarlet red	1.0	
Scarlet red sulfonate	24.0 to 27.0	
Senega		5.0
Senna		3.0
Serpentaria		10.0
Sherry wine	0.5	
Sodium alginate	18.0 to 24.0	
Squill		2.0
Sterculia gum		1.0
Stramonium		4.0
Strychnine	0.1	
Strychnine nitrate	0.1	
Strychnine sulfate	0.1	
Sulfapyridine	0.1	
Sulfur, sublimed	0.5	
Sulfur, washed	0.5	
Sulfuric acid	0.02	
Tannic acid	1.0	
Tar, pine	0.3	
Taraxacum		4.0
Terpin hydrate	0.1	
Theobromine	0.1	
Thyme		4.0
Thymol iodide	1.5	
Totaquine	5.0	
Undecylenic acid	0.15	
Veratrum viride		4.0
Viburnum opulus		2.0
Viburnum prunifolium		3.0
White pine		0.5

TABLE 38. SOME OFFICIAL SUBSTANCES WITH NONVOLATILE RESIDUE REQUIREMENTS

Substance	Amount used, Gm. or cc.	Residue dried at °C.	Official requirement, mg.
U.S.P.			
Acetic acid	20	105	1.0
Acetic acid, glacial	20	105	1.0
Alcohol	40	105	1.0
Alcohol, diluted	40	105	1.0
Amaranth solution	10	105	90.0 to 110.0
Ammonia solution, diluted	10	105	2.0
Ammonia solution, strong	10	105	2.0
Amylene hydrate	10	105	25.0
Camphor	2.0	120	1.0
Cherry juice	5.0	100, H_2SO_4, V	500
Chloroform	50.0	105	1.0
Ether	50.0	105	1.0
Ethyl chloride	5.0	evap.	negligible
Ethylenediamine solution	5	105	1.0
Glycerin	50	ignite	5.0
Hydriodic acid, diluted	5	ignite	100
Hydrochloric acid	20	ignite	2.0
Hydrochloric acid, diluted (see Hydrochloric acid)			
Hydrogen peroxide solution	20	105	30.0
Iodine	....	105	(0.50 per cent)
Menthol	2	105	1.0
Myristica oil (East Indian)	3	100	60.0
Myristica oil (West Indian)	3	100	50.0
Orange flower water	100	105	15.0
Paraldehyde	5	105	3.0
Petroleum benzin	50	40	1.0
Phenol	5	105	2.5
Phenol, liquefied (see Phenol)			
Raspberry juice	5	H_2SO_4, V(18)	250*
Rose water, stronger	100	105	15.0
Tetrachloroethylene	50	105	1.0
Trichloroethylene	50	105	1.0
Vinyl ether	10	105	2.0
Water	100	105	100.0

TABLE 38. SOME OFFICIAL SUBSTANCES WITH NONVOLATILE RESIDUE REQUIREMENTS. (*Continued*)

Substance	Amount used, Gm. or cc.	Residue dried at °C.	Official requirement, mg.
U.S.P.			
Water, distilled	100	105	100.0
Water, distilled, sterile (see Distilled water)			
Water for injection (see Distilled water)			
N.F.			
Acetic acid, diluted	20	105	1.0
Acetone	50	105	2.0
Alcohol, dehydrated	40	105	1.0
Alcohol rubbing compound	25	105	89.0*
Ammonium acetate solution	20	ignite	3.0
Aromatic solution, alkaline	10	ignite	223 to 273
Beef extract	10	105	750.0*
Brandy	20	105	300
Carbon tetrachloride	50	100	1.0
Ether spirit	25	100	1.0
Ethyl acetate	....	105	(0.02 per cent)
Formic acid	20	105	2.0
Hamamelis water	100	100	25.0
Isopropyl alcohol	50	105	2.5
Isopropyl alcohol rubbing compound	50	105	50.0
Methylrosaniline chloride solution	10	100	85.0 to 115.0
Nitric acid	30	ignite	2.0
Nitrohydrochloric acid	10	105	3.5
Nitrohydrochloric acid, diluted	20	105	2.0
Parachlorophenol	1	105	1.0
Sherry wine	25	ignite	125.0
Sherry wine	25	105	1,750
Sulfuric acid	5	ignite	1.0
Thymol	2	100	1.0
Turpentine oil, rectified	5	100	15.0

* Lower limit. Example: 89* = not less than 89 mg.

V = vacuum desiccator or reduced pressure.

method, which was developed by the U.S. Forestry Service, is commonly known as the *xylene method*, although kerosene, toluene, and other organic solvents have been employed in the place of xylene. A modification of this method is official as the *moisture method by toluene distillation*. It is the method specified for the determination of the moisture content of thyroid and is generally applicable to moisture determinations in glandular products and vegetable drugs containing 2 or more per cent of moisture. This method has the disadvantage that a comparatively large amount of drug, in some cases 50 to 100 Gm., must be used for the determination in order to secure a volume of water that can be measured conveniently without considerable error.

Most of the official chemical compounds are relatively stable in respect to their moisture content at room temperatures; *i.e.*, they neither gain nor lose more than slight traces of water. Some of them are hygroscopic and absorb water readily, *e.g.*, aluminum chloride. Others lose a part or all of their water of crystallization unless properly preserved, *e.g.*, copper sulfate. Control of the amount of water contained in chemicals is important in making solutions of definite concentration and in the determination of the dosage of those substances used as medicaments.

The moisture content of chemicals is determined by drying them to constant weight, at a specified temperature. The U.S.P. and the N.F. specify the allowable per cent of moisture in many chemicals; when no limit is specified, 5 per cent of moisture is permitted. For certain chemicals, a limit of tolerance for loss of water of crystallization has been adopted.

Exercise 66

Object. To Determine the Moisture Content of Acacia.

Materials Required. 10 Gm. of Acacia.

Procedure. 1. Powder the acacia in a mortar.

In the case of other unground or unpowdered drugs, prepare about 10 Gm. of the official sample by cutting, or shredding, so that the parts are about 3 mm. in thickness. Seeds or fruits smaller than 3 mm. should be cracked. High-speed mills, since they generate considerable heat, should not be used for preparing the sample, and care should be taken that no appreciable amount of moisture is lost during the preparation and that the portion taken is representative of the official sample.

The sample should be ground in order to expose as large a surface of the drug as possible so that the moisture may be driven off readily.

2. Accurately weigh about 10 Gm. of the drug, as prepared in a tared evaporating dish. Dry at 100° for 5 hr., and weigh. Continue the drying and weighing at 1-hr. intervals until the loss is not more than 0.25 per cent in 1 hr.'s drying.

The moisture and any other volatile constituents of acacia are driven off.

Calculate the per cent of moisture contained in the sample of acacia used.

Questions and Problems

1. Why is the loss in weight of a drug dried at 100° not always a true measure of its moisture content?

2. If an air-dried drug containing 10 per cent of moisture yielded 2.5 per cent ash calculated on the basis of the air-dried drug, what per cent of ash would the moisture-free drug contain?

Exercise 67

Object. Determination of the Moisture Content of Digitalis by the Toluene Distillation Method.

Materials Required. 50 Gm. of digitalis leaf.

200 cc. of toluene previously shaken with water, separated from any excess water, and distilled.

Toluene Moisture Apparatus. Use a 500-cc. flask preferably of Pyrex glass, a straight-tube Liebig condenser of about 500-mm. length, and a moisture-tube receiver calibrated to 0.1 cc. of the type illustrated (Fig. 18). Clean the condenser and moisture tube with cleaning mixture, rinse with distilled water and then with alcohol, and dry them in an oven at 100°.

Procedure. 1. "Place in the dry flask a quantity of the substance, weighed accurately to the nearest centigram, which it is estimated will yield from 2 to 4 cc. of water. If the substance is of a pasty character, it is best weighed in a boat of metal foil of a size that will just pass through the neck of the flask. If the substance is likely to cause bumping, add enough dry, washed sand to cover the bottom of the flask, or a number of capillary melting-point tubes, about 100 mm. in length, sealed at the upper end. Place about 200 cc. of toluene in the flask, connect the apparatus as illustrated, and fill the receiving tube *E* with toluene poured through the top of the condenser. Heat the flask gently for 15 minutes, and when the toluene begins to boil, distil at the rate of about 2 drops per second until most of the water has passed over; then increase the rate of distillation to about 4 drops per second."

Sufficient drug must be taken to yield from 2 to 4 cc. of water because smaller amounts would be difficult to determine without a relatively large per cent of error. Thus, an error of 0.05 cc. in the calibration or reading of the moisture tube would cause an error of 5 per cent if only 1 cc. of water is obtained and of 1.25 per cent if 4 cc. of water is obtained. At the boiling point of toluene, 110 to 111°, the moisture in the drug is volatilized. The moisture distills with the toluene, and as the vapors condense and drop into the moisture tube, the water, which is immiscible with and heavier than toluene, separates and collects at the bottom of the moisture tube.

2. "When the water has apparently all distilled over, rinse the inside of the condenser tube with toluene while brushing down the tube with a tube brush attached to a copper wire and saturated with toluene. Continue the distillation for 5 minutes, then remove the heat and allow the receiving tube to cool to room temperature. If any droplets of water adhere to the walls of the receiving tube, force them down with a rubber band wrapped around a copper wire and wet with toluene. When the water and toluene have separated completely, read the volume of water and calculate the percentage that was present in the substance."

Practically all the moisture distills over during the first 4 or 5 min. of distillation. The condenser tube is washed down with toluene to remove water that may adhere to its inner wall. If the condenser is cleaned as directed, little difficulty will be experienced with water adhering to the wall of the tube. Some drugs hold their moisture quite tenaciously. Consequently, it is always well to continue distillation for 2 or 3 min. after it is apparent from the test that practically all the moisture has distilled. Droplets adhering to the wall of a properly cleaned moisture tube can be dislodged by rotating the tube between the fingers or by tapping the tube gently on wood or some other base. Volatile organic substances, such as volatile oils and amines, remain dissolved in the toluene and so are not calculated as moisture. Since the result is read directly in cubic centimeters, it is apparent that the per cent of moisture would be

2 × number of cubic centimeters of water when a 50-Gm. sample is used.

Questions and Problems

1. Consult Table 39 and list five vegetable drugs, three glandular products, and five chemical compounds the moisture contents of which might be determined by the toluene distillation method.

2. Less than 1 part of water is soluble in 10,000 parts of toluene at 25°. Would the water dissolved in the toluene when moisture is determined in a drug by the toluene distillation method be appreciable if 100 cc. of toluene unsaturated with water is employed?

Certain other substances have definite limitations on the amounts of volatile matter which they will lose when heated; *e.g.*, when ignited: the loss in weight (per cent) is given in parentheses: **U.S.P.** calamine (2.0), calcium phosphate, dibasic (24.5 to 26.5), magnesium oxide (10.0), magnesium sulfate (34.0), siliceous earth (10.0), talc (5.0), zinc oxide (2.0); **N.F.** calcium phosphate, tribasic (8.0), ferric oxide, red (3.0), ferric oxide, yellow (12.0), kaolin (15.0), lead monoxide (4.0), lime (10.0), magnesium hydroxide (30.0 to 33.0), magnesium phosphate, tribasic (20.0 to 27.0), neocalamine, prepared (1.0), titanium dioxide (0.5). The loss in weight in these cases cannot be considered as moisture only, since adsorbed gases as well as other volatile matter are driven off.

TABLE 39. OFFICIAL SUBSTANCES WITH MOISTURE LIMITS

Substance	Temperature, °C., and other conditions	Moisture limit, per cent
U.S.P.		
Acacia	105 (5)	15.0
Acetanilid	H_2SO_4 (2)	0.5
Acetophenetidin	60 (1)	0.5
Acetylsalicylic acid	H_2SO_4 (5)	0.5
Agar	105 (5)	20.0
Aloe	105 (5)	12.0
Aminopyrine	60 (2)	1.0
Ammonium chloride	H_2SO_4 (4)	0.5
Amphetamine sulfate	105 (2)	1.0
Antimony sodium thioglycollate	105 (2)	2.0
Apomorphine hydrochloride	105 (2)	3.5
Atropine sulfate	105 (4)	4.0
Barbital	105 (2)	1.0
Barbital sodium	105 (3)	1.0
Bentonite	105 (2)	5.0 to 8.0
Benzalkonium chloride	K.F.M.	15.0
Bishydroxycoumarin	105 (2)	0.5
Bismuth subcarbonate	105 (3)	2.0
Bismuth subsalicylate	105 (3)	3.0
Caffeine, anhydrous	80 (4)	0.5
Caffeine, hydrate	80 (4)	8.0
Caffeine and sodium benzoate	80 (4)	3.0
Calcium carbonate, precipitated	180 (4)	2.0
Calcium lactate	120 (4)	25.0 to 30.0
Calcium mandelate	105 (4)	1.0
Carbachol	105 (2)	2.0
Carbarsone	80 (6)	1.5
Cellulose, oxidized	V, P_2O_2 (18)	15.0
Chloroazodin solution	T.D.M.	0.3
Chloroguanide hydrochloride	105 (2)	0.3
Chloroquine phosphate	105 (2)	2.0
Cholesterol	60 (4)	0.3
Cocaine	H_2SO_4 (3)	1.0
Cocaine hydrochloride	H_2SO_4 (3)	1.0
Codeine sulfate	105 (3)	12.0
Colchicine	105 (3)	3.0
Congo red	105 (4)	3.0

TABLE 39. OFFICIAL SUBSTANCES WITH MOISTURE LIMITS. *(Continued)*

Substance	Temperature, °C., and other conditions	Moisture limit, per cent
U.S.P.		
Dextrose	105 (6)	8.0 to 10.0
Dichlorophenarsine hydrochloride	V, P_2O_5 (24)	0.5
Diethylstilbestrol	105 (4)	0.5
Digitalis	T.D.M. or 100	6.0
Digitoxin	105 (2)	1.0
Digoxin	105, V (1)	0.5
Dihydromorphinone hydrochloride	105 (2)	1.5
Diiodohydroxyquinoline	H_2SO_4 (4)	0.5
Diphenhydramine hydrochloride	105 (3)	0.5
Diphenylhydantoin sodium	105 (4)	2.5
Emetine hydrochloride	105 (2)	8.0 to 14.0
Ephedrine hydrochloride	105 (3)	2.0
Ephedrine sulfate	105 (3)	2.0
Epinephrine	V, H_2SO_4 (18)	2.0
Ergonovine maleate	H_2SO_4 (4)	2.0
Ethyl aminobenzoate	H_2SO_4 (3)	1.0
Ethylmorphine hydrochloride	110, V P_2O_5 (4)	10.0
Fluorescein sodium	105 (4)	7.0
Folic acid*	70.0 to 75.0, V	10.0
Gentian	T.D.M.	15.0
Glucose, liquid	80, V	21.0
Glyceryl triacetate	T.D.M.	0.3
Heparin sodium	60, P_2O_5	12.0
Histamine phosphate	105 (2)	1.5
Homatropine hydrobromide	105 (2)	1.5
Homatropine methylbromide	105 (3)	0.5
Iodoalphionic acid	H_2SO_4 (4)	0.5
Iodochlorhydroxyquin	H_2SO_4 (4)	0.5
Lanatoside C	H_2SO_4, V	7.5
Menadione	H_2SO_4 (4)	0.3
Menadione sodium bisulfite	100, V (3)	11.0 to 16.0
Meperidine hydrochloride	H_2SO_4 (4)	1.0
Meralluride	105 (3)	3.0
Methacholine chloride	105 (4)	1.5
Mercuric oxide, yellow	105 (2)	2.0

* Pressure not more than 15 mm.

Table 39. Official Substances with Moisture Limits. *(Continued)*

Substance	Temperature, °C., and other conditions	Moisture limit, per cent
U.S.P.		
Mercurophylline	105 (4)	7.0
Mercury bichloride	H_2SO_4 (4)	1.0
Mersalyl	H_2SO_4 (18)	7.0
Methacholine chloride	105 (4)	1.5
Methamphetamine hydrochloride	105 (2)	0.5
Methenamine	H_2SO_4 (4)	2.0
Methiodal sodium	105 (4)	1.0
Morphine sulfate	130 (6)	12.0
Naphazoline hydrochloride	105 (2)	0.5
Neocincophen	H_2SO_4 (4)	2.0
Neostigmine bromide	105 (3)	2.0
Neostigmine methysulfate	105 (3)	1.0
Nicotinamide	H_2SO_4 (3)	1.0
Nicotinic acid	105 (1)	1.0
Ouabain	130 (3)	18.0 to 22.0
Oxophenarsine hydrochloride	P_2O_5, V (24)	1.0
Papaverine hydrochloride	105 (2)	1.0
Para-aminobenzoic acid	105 (2)	0.2
Penicillin G Potassium	100 (2)	1.5
Pentaquine phosphate	105 (2)	0.5
Pentobarbital sodium	90 (6)	5.0
Pentylenetetrazol	H_2SO_4 (18)	0.2
Phenobarbital	105 (2)	1.0
Phenobarbital sodium	150 (4)	7.0
Phenolphthalein	H_2SO_4 (4)	1.0
Phenolsulfonphthalein	H_2SO_4 (4)	1.0
Phenylephrine hydrochloride	105 (1)	1.0
Phthalysulfathiazole	105 (4)	2.0
Physostigmine salicylate	H_2SO_4 (4)	2.0
Pilocarpine hydrochloride	105 (1)	3.0
Pilocarpine nitrate	H_2SO_4 (4)	2.0
Piperocaine hydrochloride	105 (3)	1.0
Potassium bromide	105 (4)	1.0
Potassium chloride	105 (2)	1.0
Potassium iodide	105 (4)	1.0
Potassium nitrate	105 (4)	1.0
Potassium permanganate	H_2SO_4 (18)	0.5

TABLE 39. OFFICIAL SUBSTANCES WITH MOISTURE LIMITS. (*Continued*)

Substance	Temperature, °C., and other conditions		Moisture limit, per cent
U.S.P.			
Procaine hydrochloride	H_2SO_4	(4)	1.0
Propylthiouracil	105	(2)	0.5
Quinacrine hydrochloride	105	(4)	6.0 to 8.0
Quinidine Sulfate	120	(3)	5.0
Quinine dihydrochloride	105	(2)	3.0
Quinine hydrochloride	105	(2)	10.0
Quinine sulfate	105	(3)	5.0
Resorcinol	H_2SO_4	(4)	1.0
Riboflavin	105	(2)	1.5
Saccharin	105	(2)	1.0
Saccharin sodium	120	(4)	15.0
Scopolamine hydrobromide	105	(3)	13.0
Soap, soft, medicinal	T.D.M.		52.0
Soda lime	105	(2)	19.0
Sodium benzoate	105	(4)	1.5
Sodium biphosphate	60	(1)	
	105	(3)	10.0 to 15.0
Sodium bromide	105	(4)	1.0
Sodium citrate	150	(18)	10.0 to 13.0
Sodium iodide	120	(2)	5.0
Sodium iodomethamate	V, H_2SO_4	(24)	1.0
Sodium nitrite	H_2SO_4	(4)	1.0
Sodium phosphate	105	(4)	43.0 to 50.0
Sodium phosphate, exsiccated	105	(4)	5.0
Sodium salicylate	105	(4)	0.5
Sodium stearate	105	(4)	5.0
Sodium sulfate	105	(4)	51.0 to 57.0
Starch	120	(4)	14.0
Stibophen*	V, 120	(4)	15.0
Storax	105	(2)	20.0
Succinylsulfathiazole	105	(4)	7.0
Sulfadiazine	105	(2)	0.5
Sulfadiazine sodium	105	(2)	0.5
Sulfaguanidine	105	(4)	6.0 to 8.0
Sulfamerazine	105	(2)	0.5
Sulfamerazine sodium	105	(4)	2.5

* Pressure not more than 5 mm.

TABLE 39. OFFICIAL SUBSTANCES WITH MOISTURE LIMITS. *(Continued)*

Substance	Temperature, °C., and other conditions		Moisture limit, per cent
U.S.P.			
Sulfamerazine sodium, monohydrate	105	(4)	7.0
Sulfanilamide	105	(2)	0.5
Sulfathiazole	105	(2)	0.5
Sulfathiazole sodium	105	(4)	6.0 to 9.0
Sulfathiazole sodium, sterile	105	(2)	0.2
Sulfobromophthalein sodium	105	(3)	5.0
Sulfur, precipitated	K.F.M.		0.5
Suramin sodium	150		12.0
Tartaric acid	H_2SO_4	(3)	0.5
Tetracaine hydrochloride	H_2SO_4	(4)	1.0
Theobromine and calcium salicylate	105	(4)	5.0
Theophylline	105	(4)	9.5
Thiamine hydrochloride	105	(2)	5.0
Thiopental sodium	80	(4)	2.0
Thiopental sodium, sterile	80	(4)	2.0
Tribromoethanol	H_2SO_4	(4)	1.0
Trimethadione	H_2SO_4	(6)	0.1
Tripelennamine hydrochloride	105	(3)	1.0
Tryparsamide	105	(4)	2.5 to 3.5
Tubocurarine chloride	V, 60 P_2O_5	(4)	11.5
Thyrothricin	105	(3)	5.0
Urethan	K.F.M.		1.0
Vanillin	H_2SO_4	(4)	1.0
Vitamin B_{12}	V, 105	(2)	12.0
Wool fat	100		0.5
Wool fat, hydrous	100		25.0 to 30.0
Yeast, dried	100	(5)	7.0
N.F.			
Acetarsone	105	(1)	2.0
Acriflavine	105	(2)	8.0
Acriflavine hydrochloride	105	(1)	7.0
Alum, exsiccated	200	(4)	10.0
Aminoacetic acid	105	(2)	0.2
Ammonium iodide	105	(2)	5.0
Amobarbital	H_2SO_4	(4)	1.0
Amobarbital sodium	105	(4)	1.0

TABLE 39. OFFICIAL SUBSTANCES WITH MOISTURE LIMITS. *(Continued)*

Substance	Temperature, °C., and other conditions		Moisture limit, per cent
N.F.			
Anthralin	H_2SO_4	(4)	0.5
Antipyrine	60	(2)	1.0
Arecoline hydrobromide	80	(2)	1.0
Arsenic trioxide	105	(2)	1.0
Bismuth subgallate	105	(3)	7.0
Bismuth subnitrate	105	(2)	3.0
Brucine sulfate	105	(3)	13.0
Caffeine and sodium salicylate	80	(4)	3.0
Citrated caffeine	80	(4)	5.0
Calcium glycerophosphate	130	(5)	12.0
Calcium hypophosphite	105	(1)	3.0
Calcium iodobehenate	105	(2)	2.0
Calcium levulinate	60	(5)	10.5 to 12.0
Cantharides			10.0
Carmine	105	(2)	25.0
Charcoal, activated	120	(4)	15.0
Charcoal, animal, purified	105	(2)	12.0
Chondrus extract	65	(5)	6.0
Cinchonidine sulfate	105	(2)	8.0
Cinchonine sulfate	105	(2)	5.0
Cinchophen	105	(1)	2.0
Codeine	80	(4)	6.0
Dehydrocholic acid	105	(3)	1.0
Dibucaine hydrochloride	80, V, P_2O_5	(5)	1.0
Dioctyl sodium sulfosuccinate	105	(4)	2.5
Ergot	105	(3)	8.0
Ergot, prepared	105 or T.D.M.		6.0
Ferric cacodylate	105	(2)	5.0
Ferric glycerophosphate	130	(4)	12.0
Ferric hypophosphite	105	(2)	3.0
Ferrous gluconate	105	(4)	10.0
Glutamic acid hydrochloride	80	(4)	0.5
Halazone	H_2SO_4	(4)	0.5
Histidine monohydrochloride	130	(12)	9.0
Hydroxystearin Sulfate	T.D.M.		25.0
Ichthammol	105	(6)	50.0
Iodoform	H_2SO_4	(4)	1.0
Ipomea resin	105	(2)	4.0

TABLE 39. OFFICIAL SUBSTANCES WITH MOISTURE LIMITS. *(Continued)*

Substance	Temperature, °C., and other conditions		Moisture limit, per cent
N.F.			
Jalap resin	105	(2)	4.0
Lithium bromide	150	(4)	10.0 to 15.0
Liver concentrate	60, V	(2)	10.0
Liver, desiccated	60, V	(2)	6.0
Liver Fraction 1	60, V	(2)	35.0
Liver Fraction 2	60, V	(2)	6.0
Magnesium hydroxide	105	(2)	2.0
Mandelic acid	H_2SO_4	(4)	1.0
Manganese glycerophosphate	105	(4)	12.0
Manganese hypophosphite	105	(1)	2.0
Merbromin	105	(5)	5.0
Mercuric iodide, red	105	(3)	2.0
Mercuric oxide, red	120	(3)	1.0
Mercuric succinimide	105	(1)	2.0
Mercurous chloride, mild	H_2SO_4	(5)	0.5
Mercurous iodide, yellow	H_2SO_4	(4)	2.0
Methylcellulose	105	(1)	5.0
Methylene blue	105	(18)	18.0
Methylrosaniline chloride	105	(4)	7.5
Morphine hydrochloride	105	(3)	15.0
Neoarsphenamine	V, P_2O_5		1.5
Nitromersol	105	(2)	1.0
Pamaquine naphthoate	105	(6)	4.0
Pectin	105	(2)	10.0
Phenacaine hydrochloride	105	(6)	7.0
Phenothiazine	H_2SO_4	(6)	1.0
Phenyl salicylate	H_2SO_4	(4)	1.0
Potassium carbonate	180	(4)	10.0 to 16.5
Potassium citrate	180	(4)	3.0 to 6.0
Potassium guaicolsulfonate	105	(2)	3.0
Potassium hypophosphite	105	(1)	5.0
Potassium mercuric iodide	120	(4)	4.0
Potassium sodium tartrate	150	(3)	21.0 to 26.0
Potassium thiocyanate	105	(4)	5.0
Proflavine dihydrochloride	105	(2)	13.0
Proflavine sulfate	105	(2)	6.0
Quinine	105	(2)	15.0
Quinine bisulfate	105	(3)	24.0

TABLE 39. OFFICIAL SUBSTANCES WITH MOISTURE LIMITS. (*Continued*)

Substance	Temperature, °C., and other conditions		Moisture limit, per cent
N.F.			
Quinine hydrobromide	105	(2)	5.0
Racephedrine hydrochloride	105	(3)	2.0
Resorcinol monoacetate	100		2.5
Rutin*	V, 125	(16)	5.5 to 9.0
Salicin	H_2SO_4	(2)	2.0
Salicylanilide	80	(2)	0.5
Soap, hard	105	(3)	36.0 (unpowdered) 10.0 (powdered)
Sodium acetate	120	(4)	36.0 to 41.0
Sodium alginate	105	(4)	15.0
Sodium carbonate, monohydrated	105	(4)	10.0 to 15.0
Sodium hypophosphite	105	(1)	3.0
Sodium indigotindisulfonate	105	(2)	10.0
Sodium propionate	105	(1)	5.0
Sodium thiocyanate	105	(4)	5.0
Sodium thiosulfate	40 to 50		
	105		32.0 to 37.0
Sterculia gum	105	(5)	20.0
Strontium bromide	165	(16)	32.0
Strontium salicylate	H_2SO_4	(2)	3.0
Strychnine sulfate	105	(6)	11.5
Sulfapyridine	105	(4)	0.5
Sulfapyridine sodium, sterile	105	(4)	5.0 to 7.0
Sulfarsphenamine	V, P_2O_5		1.5
Tannic acid	105	(2)	12.0
Theobromine and sodium salicylate	105	(2)	10.0
Thimerosal	H_2SO_4		0.5
Thymol iodide	H_2SO_4	(4)	2.0
Titanium dioxide	105	(3)	0.5
Totaquine	105	(4)	5.0
Zinc undecylenate	105	(2)	1.25

* Not more than 3 mm. pressure.

Time of drying, in hours, is indicated by number in parentheses.

H_2SO_4 or P_2O_5 = dried over sulfuric acid or phosphorous pentoxide.

T.D.M. = Toluene distillation method.

V = vacuum desiccator or reduced pressure.

K.F.M. = Karl Fischer method.

Chapter 12

EXTRACTIVE AND CRUDE FIBER CONTENT

The amount of extractive that a drug yields to a given solvent is often an approximate measure of the amount of a certain constituent or group of related constituents that the drug contains. In cases where this is the object sought, it is important that a solvent be used which will not dissolve appreciable quantities of substances other than those sought in the extraction. In some cases, the amount of a drug soluble in a given solvent is an index of its purity; *e.g.*, acacia must yield not more than 1 per cent of water-insoluble residue to meet the official requirement.

For efficient and complete extraction, the solvent should be divided into a number of relatively small portions and used successively. Each portion should be brought into intimate contact with all parts of the sample and then be removed before the addition of the next portion. The use of fresh portions of the solvent that are not saturated with extractive favors the rapid and thorough removal of the solute with the minimum quantity of solvent.

The complete removal of extractive matter from a drug by this method, however, requires a large quantity of solvent in most cases, and most volatile solvents are expensive. Consequently, it is convenient and economical to be able to use the same portion of solvent repeatedly. To do so necessitates the separation of the solvent and solute after the extraction, the separated solvent being employed in a second extraction. This is readily accomplished by means of a special form of extraction apparatus of which the Soxhlet is a good example.

The Soxhlet (Fig. 19) is the apparatus generally used for extraction with volatile solvents where small quantities of drug are extracted as in quantitative work. The solvent is placed in a weighed flask *A*, which is heated over an electric hot plate or over a ater bath in the absence of a gas flame when the solvent is highly nflammable. The weighed sample of drug is placed in a porous aper extraction thimble, which is then fitted into the extraction ube *B*. A reflux condenser *C* is attached to the top f the extraction tube. The solvent vapors pass pward from the flask *A* through the side tube *D* f the extractor to the condenser. The condensate rips into the thimble until a sufficient amount has ccumulated to raise the level of liquid in the extracion tube to the top of the siphon tube *E*. The olution that has been standing in contact with the rug is then discharged into the flask where the solent is vaporized again. The solvent passes through his cycle repeatedly, and the solute is collected in he flask. The heat should be so adjusted that the olvent passes around the cycle about once every 10 nin. The top of the thimble should be above the evel of the top of the siphon side tube. The solvent nust not be volatilized more rapidly than it will filter hrough the porous thimble, or the latter will overlow and carry insoluble matter over mechanically. After the extraction is complete, the flask is removed, he solvent is driven off until the flask and its contents are of constant veight, and the percentage of extractive in the drug is calculated rom the weight of the residue.

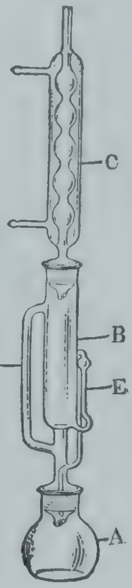

FIG. 19. Soxhlet extraction apparatus.

In some cases, it is advantageous to determine the insoluble esidue after extraction. The extraction thimble should be dried nd weighed accurately before placing the sample in it. After the xtraction is complete, it is dried with the residue and weighed accuately again. *Caution:* Do not place thimbles and residues satuated with ether or other inflammable or explosive solvents in an lectric oven until they have been air-dried.

Volatile and Nonvolatile Ether-soluble Extractive. The letermination of the total ether-soluble volatile constituents is pplied to those drugs which contain volatile oils, while the deternination of the nonvolatile ether-soluble constituents is applied o those drugs the active constituents of which are associated with volatile matter. In either case, the determination is only approximate.

Exercise 68

Object. To Determine the Volatile and Nonvolatile Ether-soluble Content of Myristica.

Materials Required. 2 Gm. of myristica.
50 to 100 cc. of absolute ether.
Soxhlet apparatus.

Procedure. 1. "Extract 2 Gm. of the prepared[1] drug, dried over sulfuric acid for not less than twelve hours, with absolute ether for twenty hours in a continuous extraction apparatus."

The drug is dried over sulfuric acid in a desiccator to remove the absorbed moisture. The extraction is continued for 20 hr. to ensure complete removal of the volatile oil and other ether-soluble constituents from the ground myristica. Absolute ether is used, since ordinary ether, which contains small, variable quantities of water, dissolves some tannin, sugar, etc.

TABLE 40. OFFICIAL SUBSTANCES WITH ETHER-SOLUBLE EXTRACTIVE

Substance	Nonvolatile, per cent	Total ether-soluble extractive, per cent
U.S.P.		
Cacao	10.0 to 22.0	
Cotton, purified	....	0.7
Pancreatin	....	3.0
N.F.		
Capsicum	12.0	
Ginger	....	4.5
Ginger fluidextract	....	4.5W/V
Ipomea resin	....	80.0
Linseed	30.0	
Myristica	25.0	
Thimerosal	....	0.8

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[1] "The drug is prepared for extraction by grinding, if necessary, until it passes through a No. 20 mesh sieve. If grinding is not possible, the drug is reduced to as fine a state as possible by other means."

2. "Transfer the ether solution to a tared porcelain dish and allow it to evaporate spontaneously. Then dry it over sulfuric acid during eighteen hours and weigh the total ether extract. Now heat the extract gradually up to 105° until the weight becomes constant: the loss in weight represents the volatile portion of the extract."

Since ether boils at 35°, it evaporates rapidly at ordinary temperatures without carrying off more than negligible amounts of the volatile extractive. The residue, dried for 18 hr. over sulfuric acid to get rid of the small amounts of water extracted by the ether, represents the ether-soluble extractive of the myristica. This residue consists chiefly of volatile oil and resin. When the residue is heated at 105° until of constant weight, the volatile oil is volatilized, leaving resin, coloring matter, etc., as the nonvolatile ether-soluble extractive. The difference in the weight of the total extractive and the nonvolatile residue is the volatile ether-soluble extract of myristica.

Alcohol-soluble Extractive. Alcohol is a good solvent for resinous matter. Consequently, the determination of the alcohol-soluble extractive is most frequently employed to determine approximately the amount of resin in those drugs in which the resinous matter is the important constituent. In some cases, the determination of the alcohol-soluble extractive is considered officially as an assay process, *e.g.*, asafetida and myrrh, and in other cases, the extractive upon further purification is employed in the assay, *e.g.*, jalap.

Exercise 69

Object. Assay of Benzoin.

Materials Required. 2 Gm. of benzoin.
100 cc. of alcohol.

Procedure. 1. Weigh 2 Gm. of the prepared drug into a dried and tared paper extraction thimble, using a glass-stoppered weighing bottle as the container, and place 0.1 Gm. of sodium hydroxide in the receiving flask. Extract the drug in a continuous extraction apparatus with alcohol, during 5 hours.

The alcohol dissolves the resins, benzoic acid, cinnamic acid, styrene, benzaldehyde, vanillin, esters, etc., contained in the balsamic resin. The NaOH in the receiving flask forms nonvolatile salts of the volatile acids.

2. Dry the insoluble residue at 100° for thirty minutes and weigh. Dete mine the moisture in the drug by the toluene distillation method, page 25 calculate the weight of moisture in the quantity of drug taken for the test an subtract it from the original weight of the drug taken for the test. The diffe ence between this result and the weight of the residue determined as directe above represents the amount of alcohol-soluble extractive.

Table 41. Official Substances with Alcohol-soluble Extractive Limi

Substance	Alcohol used, per cent	Extractive, per cent
U.S.P.		
Benzoin, Siam	94.9	90.0
Benzoin, Sumatra	94.9	75.0
Bismuth potassium tartrate	94.9	0.5*
Storax	94.9	70.0
Vanilla	49.5	12.0
N.F.		
Asafetida	94.9	50.0
Bismuth subgallate	94.9	0.5*
Calcium glycerophosphate	99.0	1.0*
Gamboge	94.9	65.0
Myrrh	94.9	30.0
Poplar bud	99.0	40.0
Rhubarb	49.5	30.0
Rhubarb, Indian	49.5	30.0
Sodium glycerophosphate	99.0	1.0*

* Upper limit. Example: 0.5* = 0.5 per cent.

The residue should be dried and weighed in the extraction thimble. When the extraction is complete, the thimble should be removed from the apparatus and allowed to drain and dry in air for about 5 min. to remove most of the alcohol. The residue and thimble can be dried conveniently at 100° in an electric oven. Since moisture remaining in the marc as well as the alcohol is volatilized at 100°, it is necessary to correct for the loss of moisture by a moisture determination.

Calculate the per cent of alcohol-soluble extractive assuming that the benzoin contained 3.20 per cent moisture.

Diluted Alcohol Extractive. The diluted alcohol extractive is determined in certain drugs containing diluted alcohol-soluble purtive principles, *e.g.*, rhubarb. The determination is carried out follows: Macerate about 2 Gm. of the prepared drug, accurately eighed, in about 70 cc. of diluted alcohol in a suitable flask. Shake e mixture during 8 hr. at 30-min. intervals and then allow it to and for 16 hr. without shaking. Filter and wash the flask and sidue with small portions of diluted alcohol, passing the washings rough the filter, until the filtrate measures 100 cc. Evaporate a -cc. aliquot portion of this filtrate to dryness in a suitable tared sh on a water bath and dry to constant weight at 110°. Calculate e percentage of this extractive from the weight of drug taken.

Water-soluble Extractive and Water-insoluble Residue. he determination of the water-soluble extractive content is applied those drugs of which one or more of the most important constitnts are soluble in water, *e.g.*, aloe, gambir, and kino. The deterination is performed in the same way as the determination of the luted alcohol-soluble extractive.

Exercise 70

Object. Assay of Aloe.

Materials Required. 2 Gm. of aloe.

Procedure. Proceed as directed above for the determination of diluted cohol extractive, using distilled water instead of diluted alcohol as the solvent.

Barbaloin, isobarbaloin, and other active constituents of a glysidal nature dissolve in the water, leaving resinous matter, etc., dissolved.

The **U.S.P.** requires that aloe contain not less than 50 per cent of ater-soluble extractive. Calculate the percentage of water-solue extractive contained in the sample examined and compare the sult with the official requirement. A water-soluble extractive nit is established for the following substances; minimum per cent given in parentheses: **U.S.P.** Aloe (50), Gentian (30); **N.F.** Ginger 2).

In addition to the official definitions of the *water-soluble extractive* the above substances, there are official limitations on the amounts *water-soluble substances* permissible in certain other official drugs;

e.g., the maximum per cent of water-soluble substances in the following are given in parentheses: **U.S.P.** chlorophenothane (0.05), cotton, purified (0.25), pyroxylin (0.30); **N.F.** bismuth magma (0.01) *W/V*, calcium iodobehenate (0.25), calcium phosphate, tribasic (0.5), ergot, prepared (0.01), ferric oxide, red (1.0), ferric oxide, yellow (1.0), jalap resin (0.0), magnesium phosphate, tribasic (1.5), magnesium phosphate, tribasic tablets (1.5), pumice (0.2), scarlet red (1.0), titanium dioxide (1.0).

Petroleum Benzin-soluble Extractive. Purified petroleum benzin is a good solvent for fats and fatty oils. The determination of the amount of extractive with this solvent is performed as follows:

> Extract completely about 2 Gm. of the prepared drug, accurately weighed, by subjecting it during twenty hours to the action of purified petroleum benzin in a continuous extraction apparatus. Transfer the benzin solution to a tared porcelain dish and allow it to evaporate spontaneously. Then dry it over sulfuric acid for eighteen hours and weigh. Calculate the percentage of anhydrous extractive from the weight of drug taken.

The petroleum benzin extractive is an index to the amount of fat present and serves as a check on the amount of seeds contained in colocynth pulp since the seeds contain a large amount of fatty matter. The upper limit (per cent) of the petroleum benzin extractive is given in parentheses in the following: **N.F.** colocynth (2.0), prepared ergot (5.0), and ipomea resin (3.0).

Jalap resin, N.F., is extracted with chloroform by maceration; the upper limit of chloroform-soluble substances is 32 per cent.

Crude Fiber. The crude fiber content of a drug is the residue, consisting chiefly of cellulose, that remains undissolved after successive treatment with boiling acid and alkali. The determination of crude fiber is of considerable importance in the examination of certain drugs and particularly of spices, since the commonly used adulterants consist of waste or refuse material derived from the drugs or spices themselves or from other food products. Frequently, this material is the outer cellular layer or protective coating, which contains a larger proportion of lignified tissues and, consequently, more crude fiber than the part official.

Exercise 71

Object. To Determine the Crude Fiber Content of Clove.

Materials Required. 2 Gm. of clove in fine powder.
200 cc. of 1.25 per cent H_2SO_4.
200 cc. of 1.25 per cent NaOH.

Procedure. 1. "Exhaust a weighed quantity of the prepared drug representing about 2 Gm. of the drug, with ether or use the residue from the determination of the volatile ether-soluble extractive."

The drug should be powdered as finely as possible so that the acid and alkali will penetrate the innermost tissues and thus ensure complete removal of soluble material. It is exhausted first with ether to remove fats and waxes, which, being immiscible with aqueous solutions, tend to prevent the penetration of the acid and alkali solution into the drug particles.

2: "Add 200 cc. of boiling sulfuric acid solution, adjusted to exactly 1.25 per cent by titration, to the ether-exhausted drug, in a 500-cc. flask, and connect the flask with a reflux condenser, the tube of which passes only a short distance below the rubber stopper, into the flask. Heat the mixture to boiling and continue the boiling exactly thirty minutes. Then filter through a linen or hardened-paper filter and wash the residue on the filter with boiling water until no longer acid."

By diluting exactly 51 cc. of $1N$ sulfuric acid or an equivalent amount of a standard acid of known normality to 200 cc. at 25°, 200 cc. of 1.25 per cent sulfuric acid may conveniently be prepared. The prepared acid solution should be measured and heated to boiling before it is added to the drug. When the acid solution is added to the powdered, ether-exhausted drug contained in a cold flask, the solution is cooled below its boiling point. The mixture should be heated rapidly to bring it to the boiling point again, and thereafter the flame should be cut down to the minimum required to maintain slow, steady boiling. Since the amount of material dissolved by the acid solution is partly dependent upon the length of time that the latter is heated in contact with the sample, it is important that the mixture should be boiled exactly 30 min., the time period being taken from the time the mixture starts to boil and not from the time the hot acid solution is added. The flask should be rotated gently from time to time while the mixture is being boiled to bring particl of the drug that adhere to the walls of the flask into contact with th acid. A current of air, passed into and beneath the surface of th mixture, through a capillary tube, will frequently prevent excessiv frothing. The acid-insoluble residue is collected on a filter an washed until free of acid, since the latter, if present, would partiall neutralize and, consequently, reduce the concentration of the alka used in the next step of the procedure.

3. "Rinse the residue back into the flask with 200 cc. of boiling sodiu hydroxide solution, adjusted to exactly 1.25 per cent by titration and free fro sodium carbonate. Again heat the mixture to boiling and continue the boilin exactly 30 min. under the reflux condenser as described under the treatme with acid, then rapidly filter through a tared filter, wash the residue with boilin water until the last washing is neutral, dry it at 105° until of constant weight and note the weight."

The alkali solution may be prepared conveniently by dilutin 70 cc. of recently standardized, carbonate-free, $1N$ sodium hydroxid or an equivalent quantity of standard alkali solution of known nor mality to exactly 200 cc. The residue from the acid treatment i step 2 should be boiled with alkali in a manner duplicating as nearl as possible that used in the boiling with acid. The residue is the collected on a filter, preferably in a Gooch crucible, washed free o alkali, dried, and weighed accurately. The dried residue consist chiefly of cellulose and small amounts of hemicelluloses, pentoses inorganic matter, etc.

4. "Incinerate the dried residue, and weigh the ash."

The fibrous matter should be incinerated cautiously at first an then at a low red heat until the carbon is completely burned off, as indicated by the formation of a white ash. The cellulose, etc., is burned when the fiber is incinerated, leaving the inorganic matter. The weight of the ash obtained subtracted from the weight of the residue found in step 3 gives the weight of the crude fiber.

Questions and Problems

1. What is meant by the term *crude fiber?*

2. Why should the sample used be finely ground and exhausted with ether before it is boiled with acid and alkali?

TABLE 42. OFFICIAL SUBSTANCES WITH INSOLUBLE RESIDUE LIMITS

Substance	Solvent	Insoluble residue limit, per cent
.S.P.		
Acacia	Diluted hydrochloric acid	1.0
Acetylsalicylic acid	Sodium carbonate T.S.	0.0
Agar	Water	1.0
Aloe	Alcohol	10.0
Aloin	Water	1.5
Boric acid	Alcohol	0.0
Boric acid	Water	0.0
Calamine	Diluted hydrochloric acid	2.0
Calcium carbonate, precipitated	Diluted hydrochloric acid	0.2
Calcium hydroxide	Diluted hydrochloric acid	0.5
Calcium phosphate, dibasic	Diluted hydrochloric acid	0.1
Gauze, absorbent	Water	0.25
Gauze, absorbent, sterile	Water	0.25
Gauze, bandage	Water	0.25
Glycyrrhiza extract	Water	25.0
Magnesium carbonate	Diluted hydrochloric acid	0.05
Magnesium oxide	Diluted hydrochloric acid	0.1
Mercury bichloride	Ether	0.3
Ox bile extract	Alcohol (80 per cent)	0.1
Pentylenetetrazol	Ether	1.0
Phenolsulfonphthalein	Sodium bicarbonate solution	0.2
Potassium hydroxide	Water	0.0
Soap, soft, medicinal	Alcohol	3.0
Sodium bicarbonate	Water	0.0
Sodium phosphate	Water	0.2
Sodium phosphate, exsiccated	Water (see Sodium phosphate)	0.2
Sodium stearate	Alcohol	0.0
Storax	Alcohol	5.0
Urea	Alcohol	0.04
.F.		
Acriflavine	Water	0.5
Alum, exsiccated	Water	2.5
Antipyrine	Water	0.0
Barium chloride	Water	0.02

TABLE 42. OFFICIAL SUBSTANCES WITH INSOLUBLE RESIDUE LIMITS. (*Continued*)

Substance	Solvent	Insoluble residue limit, per cent
N.F.		
Beef extract	Alcohol	10.0
Calcium hypophosphite	Water	0.5
Calcium levulinate	Water	0.1
Calcium phosphate, tribasic	Diluted hydrochloric acid	0.2
Chalk, prepared	Diluted hydrochloric acid	2.0
Copaiba	Alcohol (99.0 per cent)	5.0
Ferric oxide, red	Diluted hydrochloric acid	1.0
Ferric oxide, yellow	Diluted hydrochloric acid	1.0
Guaiac	Alcohol	15.0
Ipomea resin	Alcohol	0.5
Iron, reduced	Diluted sulfuric acid	1.0
Lead monoxide	Acetic acid	2.0
Lime	Diluted hydrochloric acid	1.0
Magnesium phosphate, tribasic	Hydrochloric acid	0.2
Mastic	Ether	3.0
Mastic	Alcohol	20.0
Mercuric iodide, red	Potassium iodide solution	0.2
Mercuric salicylate	Sodium hydroxide, 1*N*	0.0
Methylrosaniline chloride	Alcohol	1.0
Neocalamine, prepared	Hydrochloric acid	2.0
Nitromersol	Sodium hydroxide T.S.	0.1
Phenothiazine	Ether	1.5
Potassium bitartrate	Ammonia T.S.	0.0
Potassium carbonate	Water	0.0
Soap, hard	Water	0.15
Soap, hard	Alcohol	1.0
Sodium alginate	Water	0.2
Sodium indigotindisulfonate	Water	0.5
Theobromine and sodium salicylate	Water	0.0
Trinitrophenol	Benzene	0.2
Turpentine	Alcohol	2.0

3. Why should the boiling with 1.25 per cent acid and alkali be continued for exactly 30 min.?

4. What substances remain undissolved by the acid and alkali?

In certain monographs limitations have been placed on the amount of substance that is insoluble in a given solvent and serve primarily to check the purity and identity of the substance.

The Pharmacopoeia requires that clove contain not more than 10 per cent of crude fiber. Calculate the percentage of crude fiber contained in the sample examined and compare the results with the official requirements.

A crude fiber limit is given for cacao, U.S.P. (7.0 per cent of ether-insoluble residue).

Chapter 13

CONSTANTS OF FATS, FATTY OILS, WAXES BALSAMS, RESINS, ETC.

The methods of analysis of fatty substances, waxes, resins, etc usually consist of the determination of a number of physical an chemical properties or values commonly known as *constant* although they are constant only within certain limits. These constants, when taken in conjunction with the color, odor, taste, an special identity tests for the given substance and for common adu terants, are the basis upon which the purity and quality of thes substances are judged. Although the following determination are not required for all the official substances that fall under th above caption, they are generally used in the examination of suc substances.

The specific gravity, solubility, melting point, congealing poin refractive index, and optical activity determinations are performe according to the methods which are discussed later (pages 397 to 460

Preparation of the Sample of Fats and Oils. "If a samp of oil shows turbidity owing to separated stearin, warm the container in a bath of water at 50° until the turbidity has disappeare and the oil is clear. Thoroughly mix the clarified oil before weighing the samples. If the oil does not become clear on warming, filte it through dry filter paper in a funnel contained in a hot wate jacket. Weigh at one time as many portions as are needed for th various determinations, using preferably a bottle having a pipett dropper, or a weighing burette. Keep the sample melted, if soli at room temperature, until the desired samples are withdrawn."

Acid Value. The *acid value*, also known as the *acid number* an as the *acidity index*, is defined as *the number of milligrams of potassium hydroxide necessary to neutralize the free acids in 1 Gm. of oi fat, wax, resin, balsam, or similar organic substance of complex con* *osition.* In other words, it gives the amount of potassium hydroxe, expressed in tenths of 1 per cent, required to neutralize the free ids in a substance. The acidity may also be expressed as the umber of cubic centimeters of 0.1*N* NaOH required to neutralize e free acid in 10 Gm. of substance. This value is determined by trating a weighed sample of the substance, contained in an alcoolic or in an alcohol-ether solution, with standard alkali solution, sing phenolphthalein as the indicator. For the titration, 0.5*N*, 1*N*, or 0.02*N* alkali may be employed, but a solution of 0.1*N* conntration is most suitable in the majority of the official determinaons. For substances like the balsams and resins, a mixed solvent, ch as alcohol and ether, may be used advantageously, since the loring matter that interferes with the observation of the end point dissolved in the ethereal layer when the alkali is added. Solid ts and waxes are usually melted on a water bath and titrated while ot.

The presence of free acids in the oils, fats, and waxes is due chiefly the hydrolysis of the esters composing them and is caused by emical treatment, by bacterial action, or by the catalytic action light and heat. As a rule, fresh or recently prepared fatty subances contain little or no free acids. Upon aging, the acid value creases slowly at first and more rapidly later, especially if the bstance is not well protected from the simultaneous action of ht and air. The acid values of the official fatty substances are ite variable; consequently, the official standards fix maximum nits, in most cases, which if exceeded indicate that the substances ve undergone hydrolytic decomposition in their preparation or urification, or during the period of storage. High acid values are ot necessarily an indication of rancidity, since the latter is a result the action of the air, or possibly bacteria, on the liberated fatty ids.

A minimum acid value or a minimum and a maximum acid value generally given in the official standards for substances that are lsamic or resinous in character. The value of these substances usually based, in part at least, upon their content of free acids; *g.*, the acid value of tolu is required to be not less than 112 and not ore than 168. Since a good grade of tolu usually contains from

12 to 15 per cent of cinnamic and benzoic acid in the free state, an acid value of less than 112 would indicate that the acid content of the balsam was low and that it was of inferior quality or adulterated. On the other hand, an acid value greater than 168 would indicate adulteration with some substance having high acid value, such as certain resins.

Exercise 72

Object. To Determine the Acid Value of Rosin.

Materials Required. About 1 Gm. of rosin.
0.1N sodium hydroxide.
About 50 cc. of a mixture of equal volumes of alcohol and ether.

Procedure. 1. Pulverize the rosin in a mortar and dissolve about 1 Gm. of the powder, accurately weighed, in from 40 to 50 cc. of the neutral alcohol-ether mixture.

The rosin is powdered to facilitate its solution. Commercial alcohol and ether are frequently acid in reaction, and the mixture should always be tested before the sample is dissolved. This test may conveniently be carried out in this case by adding 1 cc. of phenolphthalein indicator solution to the alcohol-ether mixture before the rosin is dissolved. If the mixture is found to be acidic, it should be rendered neutral to phenolphthalein by the addition of the standard alkali solution.

In the titration of substances that form colored solutions, the end point may frequently be made observable by dilution with alcohol to a volume of from 100 to 200 cc.

2. Add 1 cc. of phenolphthalein indicator solution (if it has not already been added in testing the neutrality of the alcohol-ether mixture) and titrate the solution with 0.1N NaOH, agitating the mixture continuously.

In the titration of fatty substances, it is necessary to shake the mixture thoroughly after the addition of each portion of alkali to secure complete extraction of the fatty acids from the immiscible oily layer. This precaution is not of great importance in the titration of rosin, however, since rosin is soluble in alcohol and ether, and also in alkalies.

The alkali reacts with the dibasic abietic acid formed from the abietic acid anhydride and with the other acids contained in the

TABLE 43. ACIDITY LIMITS OF OFFICIAL FATTY SUBSTANCES

A. Expressed in terms of acid values

Substance	Amount used, Gm.	Alkali used	Official requirement, acid value
U.S.P.			
Oleic acid	1	0.1*N* NaOH	188 to 203
Peruvian balsam	1	0.5*N* NaOH	56 to 84
Soap, soft, medicinal	1	0.1*N* NaOH	(n.m.t.) 205
Stearyl alcohol	1	0.1*N* NaOH	(n.m.t.) 2
Storax, Levant	1	0.5*N* NaOH	50 to 85
Storax, American	1	0.5*N* NaOH	36 to 85
Tolu balsam	1	0.5*N* KOH (alcoholic)	112 to 168
Wax, white	3	0.5*N* KOH (alcoholic)	17 to 24
Wax, yellow	3	0.5*N* KOH (alcoholic)	18 to 24
N.F.			
Cetyl alcohol	10	0.1*N* NaOH	(n.m.t.) 2
Glyceryl monostearate	10	0.1*N* NaOH	(n.m.t.) 18
Mastic	2	0.1*N* NaOH	(n.m.t.) 50
Oleyl alcohol	10	0.1*N* NaOH	(n.m.t.) 2
Rosin	1	0.1*N* NaOH	(n.l.t.) 150
Soap, hard (acids)	1	0.1*N* NaOH	185 to 205

B. Expressed in terms of the amount of standard alkali required for neutralization

Substance	Amount used, Gm. or cc.	Alkali used	Official requirement, cc. of alkali
U.S.P.			
Almond oil, expressed	10	0.1*N* NaOH	(n.m.t.) 5
Castor oil	10	0.1*N* NaOH	(n.m.t.) 7
Cod liver oil	2	0.1*N* NaOH	(n.m.t.) 1
Cod liver oil, nondestearinated	2	0.1*N* NaOH	(n.m.t.) 1
Corn oil	10	0.02*N* NaOH	(n.m.t.) 2
Halibut liver oil	2	0.1*N* NaOH	(n.m.t.) 1
Halibut liver oil capsules	2	0.1*N* NaOH	(n.m.t.) 1
Oleovitamin A	2	0.1*N* NaOH	(n.m.t.) 1
Oleovitamin A and D	2	0.1*N* NaOH	(n.m.t.) 1
Oleovitamin A and D, concentrated	2	0.1*N* NaOH	(n.m.t.) 1
Oleovitamin D, synthetic	2	0.02*N* NaOH	(n.m.t.) 1.5
Olive oil	10	0.1*N* NaOH	(n.m.t.) 5
Peanut oil	10	0.02*N* NaOH	(n.m.t.) 2
Persic oil	10	0.02*N* NaOH	(n.m.t.) 2
Petrolatum	20	0.1*N* NaOH	(n.m.t.) 0.4
Sesame oil	10	0.02*N* NaOH	(n.m.t.) 3
Sodium morrhuate injection	5	0.1*N* NaOH	(n.m.t.) 0.5
Sodium stearate	2	0.1*N* NaOH	0.6 to 0.85
Theobroma oil	10	0.1*N* NaOH	(n.m.t.) 5
Wool fat	10	0.1*N* NaOH	(n.m.t.) 2
Wool fat, hydrous	10	0.1*N* NaOH	(n.m.t.) 2
N.F.			
Chaulmoogra oil	10	0.1*N* NaOH	(n.m.t.) 40
Coconut oil	10	0.1*N* NaOH	(n.m.t.) 0.55
Hydroxystearin sulfate	10	0.1*N* NaOH	(n.m.t.) 98
Linseed oil	10	0.1*N* NaOH	(n.m.t.) 7.5
Lard	10	0.1*N* NaOH	(n.m.t.) 2
Suet, prepared	10	0.1*N* NaOH	(n.m.t.) 6

(n.m.t.) = not more than. (n.l.t.) = not less than.

rosin with the formation of so-called "rosin soaps" and water. I has been suggested that the acid value of rosin can be more accurately determined in alcoholic solution, using alcoholic KOH in th titration, since the presence of water in the above determinatio causes dissociation of the rosin soap with consequent low results.

The calculation of the acid value is simple; *e.g.*, if a 1-Gm. sampl of rosin required 30 cc. of $0.1N$ NaOH for titration, the acid valu would be $\frac{30 \times 5.610}{1} = 168.30$, where 5.610 is the number of milligrams of KOH equivalent to 1 cc. of $0.1N$ NaOH solution.

In addition to the listed substances for which definite acid valu limits are given in the official standards, there are a number of substances for which the maximum content of free fatty acids is fixe by the volume of standard alkali solution required in their titration

Questions and Problems

1. Define the term *acid value.*

2. Why must the alcohol or ether used as a solvent for fats, resins, etc., i acid value determinations be neutral?

3. Look up the U.S.P. test for free fatty acids under lard and explain why thi test may serve as a criterion of the purity of this fat.

4. Why do the official standards set up maximum acid values for most of th official fats and oils and minimum values for most of the resinous substances

5. If a 2-Gm. sample of cod liver oil required 4.5 cc. of $0.02N$ NaOH in th titration of the free fatty acids, would the oil conform with the official purit requirement? What would be the acid number of the oil?

6. Calculate the minimum and maximum acid values permitted under th tests for purity of chaulmoogra oil.

Saponification Value. *The saponification value, saponificatio number*, or *Koettsdorfer number*, as it is sometimes called from th originator of the process, is defined as *the number of milligrams o potassium hydroxide required to neutralize the free acids and saponif the esters contained in 1 Gm. of fat, fatty or volatile oil, wax, resin balsam, or other substance of similar composition.* This value represents the amount of potassium hydroxide, expressed in tenths o 1 per cent, required to neutralize the total free and combined acid in 1 Gm. of the substance, or, in other words, it is ten times the percentage of potassium hydroxide required to neutralize all of the acid contained in the sample after saponification. Since the natural fat and oils consist of mixtures of glyceryl esters of the higher acids, their saponification values do not differ greatly. The determination of the saponification value, however, serves to aid in the detection of the presence of the glycerides of acids containing less than 16 or more than 18 carbon atoms, since the value of this constant is inversely proportional to the mean molecular weights of the acids present. In some cases, it may also indicate adulteration of the sample with unsaponifiable matter, such as mineral oil.

Exercise 73

Object. To Determine the Saponification Value of Cottonseed Oil.

Materials Required. 2 Gm. of cottonseed oil.

50 cc. of $0.5N$ alcoholic potassium hydroxide (see page 299).

About 50 cc. $0.5N$ hydrochloric acid.

"If the oil has been saturated with carbon dioxide for the purpose of preservation, it should be exposed in a shallow dish in a vacuum desiccator for twenty-four hours before the portions are weighed for this determination."

Procedure. 1. Place from 1.5 to 2 Gm. of the sample, accurately weighed, in a flask of from 200- to 250-cc. capacity, and add to it exactly 25 cc. of alcoholic half-normal potassium hydroxide. Insert into the neck of the flask, by means of a perforated stopper, an air condenser consisting of a glass tube from 70 to 80 cm. in length and from 5 to 8 mm. in diameter, and heat the flask on a water bath for 30 min., frequently rotating the contents.

If the sample is clear, it need not, as a rule, be filtered. When the presence of extraneous matter, however, makes it necessary to filter the oil, the operation may be carried out easily by means of a suction filter. The sample of oil should be introduced into the weighed flask by means of a dropper to avoid the possibility of a portion adhering to the neck of the flask, and the whole reweighed. The long glass tube serves as an air condenser to prevent the escape of alcohol vapor. An ordinary water-jacketed condenser may be used with equally satisfactory results. The mixture of oil and $0.5N$ alcoholic KOH is heated on a water bath and mixed by imparting a rotatory motion to the flask from time to time, in order to accelerate the saponification. The alcoholic KOH solution used should not be deeply colored (see page 300), since the color would interfere with the observation of the end point in the titration of the saponification mixture. Alcoholic KOH, rather than an aqueous solution, is employed, because the oils are more soluble in alcohol than in water, and because the products of saponification are completely soluble in alcohol, whereas when aqueous solutions are used, the unsaponifiable matter remains insoluble.

When saponification is complete, the mixture should be clear and free from oil globules. Although the glyceryl esters present in fatty oils vary greatly with respect to their acid components, the character of the saponification reaction may be illustrated as follows:

$$\underset{\text{Glyceryl oleate}}{C_3H_5(C_{17}H_{33}CO_2)_3} + 3KOH \longrightarrow \underset{\text{Glycerin}}{C_3H_5(OH)_3} + \underset{\text{Potassium oleate}}{3C_{17}H_{33}CO_2K}$$

2. Then add 1 cc. of phenolphthalein T.S. and titrate the excess of potassium hydroxide with 0.5*N* hydrochloric acid.

The approximate amount of alkali consumed in the saponification of the oil is found by subtracting the volume of 0.5*N* HCl used in the residual titration from the volume of 0.5*N* alcoholic KOH originally added. Sulfuric acid should not be substituted for hydrochloric acid, since potassium sulfate would be precipitated from the alcoholic solution and this would interfere with the observation of the end point.

3. Make a blank test at the same time, using exactly the same amount of alcoholic 0.5*N* potassium hydroxide. The difference in the number of cubic centimeters of 0.5*N* hydrochloric acid consumed in the actual test and the blank, multiplied by 28.05 and divided by the weight of the sample taken, gives the saponification value.

The blank test should be carried out at the same time as the sample is run, using similar flasks, boiling for the same length of time and under similar conditions, except that the oil should be omitted. This is done to eliminate as far as possible errors from every source, such as those which would be introduced by the absorption of CO_2 by the alkali or by the alkalinity of the glass.

The saponification value may be calculated as in the following example: 1.532 Gm. of cottonseed oil saponified with 25 cc. of 0.5*N* alcoholic KOH required 11.0 cc. of 0.5*N* HCl to back-titrate the excess alkali. In the blank test 21.5 cc. of 0.5*N* HCl were required to titrate the alkali. Therefore, a quantity of potassium hydroxide corresponding to $\frac{(21.5 - 11.0)0.0561}{2} \times 1{,}000 = 294.5$ mg. of KOH was consumed in the saponification, and the number of milligrams of KOH required to saponify 1 Gm. of the oil is 294.5 1.532 = 192.2 mg. of KOH. The saponification value of the sample of cottonseed oil is therefore 192.2.

Since 1 cc. of 0.5*N* HCl is equivalent to 1 cc. of 0.5*N* KOH or to $\frac{56.10}{2 \times 1{,}000} = 0.02805$ Gm. of KOH, the saponification value may be calculated more simply from the formula:

$$\frac{\text{cc. of } 0.5N \text{ HCl} \times 28.05}{\text{wt. of sample}} = \text{saponification value, or } \frac{10.5 \times 28.05}{1.532}$$

= 192.2 mg. of KOH required to saponify the esters and neutralize the free fatty acids in 1 Gm. of the sample.

The saponification values of the official fats, fatty oils, waxes, resins, etc., are determined in the same way.

Questions and Problems

1. Define the term *saponification value.*
2. Why should a blank determination be run parallel with the determination of the saponification value of the sample?
3. Consult the following table and enumerate several oils that, if used to adulterate sesame oil, might lower its saponification value.
4. A sample of chaulmoogra oil weighing 1.600 Gm. saponified with 25 cc. of 0.4*N* KOH required 9.0 cc. of 0.5*N* HCl to titrate the excess KOH. In the blank determination, 20 cc. of 0.5*N* HCl was required to titrate the alkali. Calculate the saponification value of the sample. Does the value found correspond with the U.S.P. requirement?
5. Need the alcoholic KOH used in a saponification value determination be of an exact known normality?
6. How is the saponification value of balsam of Peru determined in the U.S.P.? Does the method correspond with that given in the preceding exercise?

Ester Value. *The ester value, or ester number*, is defined as *the number of milligrams of potassium hydroxide required to saponify the esters in 1 Gm. of a fatty or volatile oil, fat, wax, balsam, resin, or similar organic substance.* In those substances that do not contain free acids, the ester number is equal to the saponification value. When free acids are present, however, the ester value is the difference between the acid and saponification values.

TABLE 44. SAPONIFICATION VALUE LIMITS OF OFFICIAL SUBSTANCES

Substance	Amount used, Gm.	Saponification value
U.S.P.		
Almond oil, expressed	1.5 to 2	190 to 200
Castor oil	1.5 to 2	179 to 185
Cod liver oil	1.5 to 2	180 to 192
Cod liver oil, nondestearinated	1.5 to 2	180 to 192
Corn oil	1.5 to 2	187 to 193
Cottonseed oil	1.5 to 2	190 to 198
Halibut liver oil	1.5 to 2	160 to 180
Olive oil	1.5 to 2	190 to 195
Peanut oil	1.5 to 2	186 to 194
Persic oil	1.5 to 2	185 to 195
Peruvian balsam	3	230 to 240
Sesame oil	1.5 to 2	188 to 193
Storax	2	160 to 200
Theobroma oil	1.5 to 2	188 to 195
Tolu balsam	1	154 to 220
N.F.		
Chaulmoogra oil	1.5 to 2	196 to 213
Coconut oil	1.5 to 2	251 to 262
Dioctyl sodium sulfosuccinate	1.5 to 2	240 to 253
Ethyl chaulmoograte	1.5 to 2	190 to 196
Glyceryl monostearate	1.5 to 2	164 to 170
Lard	1.5 to 2	195 to 203
Linseed oil	1.5 to 2	187 to 195
Suet, prepared	1.5 to 2	193 to 200

The ester value is of particular importance in the analysis of beeswax, since it serves, in many cases, to indicate the presence of adulterants, such as paraffin. Considered in conjunction with the acid value, the ester value may aid in the detection of such adulterants as rosin and stearic acid in the wax.

The United States Pharmacopoeia gives the following directions for the determination of the ester value:

"Shake from 1.5 to 2 Gm. of the substance, accurately weighed in a 200- to 250-cc. tared flask, with from 20 to 30 cc. of neutralized alcohol, add 1 cc.

of phenolphthalein T.S., and titrate with 0.5N alcoholic potassium hydroxide until the free acid is neutralized. Add exactly 25 cc. of 0.5N alcoholic potassium hydroxide and proceed as directed under *Saponification Value*, beginning with 'Insert in the neck of the flask' and omitting the further addition of phenolphthalein T.S. The difference between the number of cubic centimeters of 0.5N hydrochloric acid consumed in the actual test and in the blank, multiplied by 28.05 and divided by the weight in Grams of the sample taken, is the Ester Value."

Questions and Problems

1. Define the term *ester number*.

2. If a sample of beeswax is found to have an acid number of 20.4 and a saponification value of 89.8, what would be the ester number of the sample?

3. If a sample of white beeswax has an acid value of 18.45 and an ester value of 74, what would be the saponification value of the sample?

Official substances with their required ester numbers include white wax 72 to 79 and yellow wax 72 to 77.

Unsaponifiable Matter. The term *unsaponifiable matter* is defined in the Pharmacopoeia as *the substances present in oils or fats that are not saponified by alkali hydroxides but are soluble in ordinary fat solvents.*

When oils and fats are saponified, there remains a small amount of residue that may consist of phytosterol in the vegetable oils and fats, of cholesterol in the animal oils and fats, and in some cases, of unsaponifiable substances added for the purpose of adulteration. The normal unsaponifiable matter present in most oils and fats dissolves in the alcoholic soap solution obtained upon saponification. When a large amount of unsaponifiable matter is present, however, it may separate out and float on the surface of the mixture.

The determination of the amount of unsaponifiable matter present in certain oils, such as linseed oil and cod liver oil, may be indicative of the quality and purity of the oil. Thus, the addition of petroleum oils, a common adulterant, to linseed oil would greatly increase the unsaponifiable-matter content of the latter.

The official compendiums give the following procedure for the determination of the amount of unsaponifiable matter in a fat or wax:

Weigh 5 Gm. of the oil or fat into a 250-cc. Erlenmeyer flask, add a solution of 2 Gm. of potassium hydroxide in 40 cc. of alcohol, and heat under a reflux condenser for 2 hours, keeping the alcohol gently boiling. Evaporate the alcohol on a water bath, dissolve the residue in 50 cc. of hot water, and transfer the solution to a separator, rinsing the flask with two 25-cc. portions of hot water which are added to the separator. Cool to room temperature, and extract with two successive portions of 50 cc. each of ether, adding a few drops of alcohol to facilitate the separation of the two liquids. Combine the ether extracts in another separator and wash the ether solution first with 20 cc. of a solution of sodium hydroxide (4 in 1,000), then with 20 cc. of a solution of sodium hydroxide (8 in 1,000), and finally with 15-cc. portions of water until the last washing is not reddened by the addition of 2 drops of phenolphthalein T.S. Transfer the ethereal solution to a tared beaker, and rinse the separator with 10 cc. of ether, adding the rinsings to the beaker. Evaporate the ether just to dryness on a water bath, and dry the residue for 30 minutes at 100°. Cool the beaker in a desiccator for 30 minutes, and weigh the residue of Unsaponifiable Matter.

The Pharmacopoeia requires that cod liver oil and nondestearinated cod liver oil shall contain (n.m.t.) 1.3 per cent; corn oil, (n.m.t.) 1.5 per cent, and halibut liver oil, (n.l.t.) 7 per cent and (n.m.t.) 22.5 per cent of unsaponifiable matter. Linseed oil, N.F. must contain (n.m.t.) 1.5 per cent unsaponifiable matter.

Iodine Value. *The iodine value, or number, is the number of grams of iodine absorbed by* 100 *Gm. of oil, fat, wax, or other substance under specified conditions.* This value is a quantitative measure of the proportion of unsaturated fatty acids present, both free and combined as esters, that have the property of absorbing iodine.

The determination of the iodine number of fats and oils is important, since it serves to characterize them and to indicate whether they are pure or admixtures. The so-called "drying oils," such as linseed oil, and the fish oils, such as cod liver oil, have very high iodine numbers, usually above 120, since they contain a large proportion of unsaturated fatty acids; the nondrying oils, such as olive oil and almond oil, have relatively low iodine numbers, below 100; and the semidrying oils, such as cottonseed oil and sesame oil, have intermediate iodine values, that is, between 100 and 120. In the case of the animal fats, the iodine number is not very high, usually being less than 90. The determination of the iodine number, therefore, not only serves as an aid to the identification of known oils, but it also serves to indicate in a definite manner the class to which an unknown fat or oil belongs. Furthermore, when the iodine number is considered in conjunction with the saponification value of a fat or oil, it serves as a means of detecting adulteration, and frequently it indicates the nature of the adulterant; *e.g.*, olive oil might be adulterated with cottonseed oil without changing the saponification value appreciably, but the iodine number of the olive oil would be increased. Again, castor oil might be adulterated with olive oil without changing the iodine number greatly, but the saponification value of the castor oil would be increased.

Several methods have been developed for the determination of the iodine number of fats and oils. These methods are generally designated by the name of their originators, as, for example, the Hübl, Hanus, and Wijs methods. The method given in the Pharmacopoeia is essentially that of Hanus. In this method, the following solutions are required:

1. *Iodobromide Test Solution.* This solution is prepared as follows:

> Dissolve 13.2 Gm. of reagent iodine in 1,000 cc. of glacial acetic acid with the aid of gentle heat, if necessary. Cool the solution to 25° and determine the iodine content in 20 cc. by titration with $0.1N$ sodium thiosulfate. Add to the remainder of the solution a quantity of bromine equivalent to that of the iodine present. Preserve in glass-stoppered bottles, protected from light.

No difficulty will be experienced in dissolving the iodine, as a rule, if the glacial acetic acid contains less than 0.5 per cent of water. The concentration of this solution is determined by titration with $0.1N$ sodium thiosulfate (see page 190). Thus, if 20.5 cc. of the iodine solution requires 20 cc. of $0.1N$ sodium thiosulfate, each cubic centimeter of the iodine solution must contain $\frac{20}{20.5} \times 0.01269 = 0.01233$ Gm. of iodine, and the remaining 980 cc. of iodine test solution would contain $980 \times 0.01233 = 12.05$ Gm. of iodine. Since 126.90 Gm. of iodine is equivalent to 79.92 Gm. of bromine, the quantity of bromine equivalent to the iodine contained in the solution may be calculated.

$$X:12.05::79.92:126.92$$
$$X = 7.59 \text{ Gm. of bromine}$$

Since bromine has a specific gravity of about 3.1, 7.64 Gm. of bromine is equivalent to 7.64/3.1 = 2.5 cc. in round numbers. Inasmuch as a slight excess of iodine does no harm, the addition of 2.25 cc. of bromine to the iodine solution would be sufficient in this case.

The bromine may be drawn into a measuring pipette by means of a rubber bulb and then added to the cool iodine solution. (Never draw bromine into a pipette by means of the mouth.)

2. *Potassium iodide test solution*, prepared by dissolving 16.5 Gm. of potassium iodide in sufficient water to make 100 cc.

3. *Tenth-normal sodium thiosulfate solution*, prepared and standardized as directed on page 187.

4. *Starch test solution*, prepared as follows:

Triturate 1 Gm. of arrowroot starch with 10 cc. of cold water, and pour slowly, with constant stirring, into 200 cc. of boiling water. Boil the mixture until a thin, translucent fluid is obtained. (Longer boiling than necessary renders the solution less sensitive.) Allow to settle and use only the clear supernatant liquid. This test solution must be freshly prepared.

Exercise 74

Object. To Determine the Iodine Value of Olive Oil.

Materials Required. About 1 Gm. of olive oil.
20 cc. of chloroform.
50 cc. of iodobromide test solution.
60 cc. of potassium iodide test solution.
About 100 cc. of 0.1N sodium thiosulfate solution.
Starch test solution.

Procedure. 1. Introduce about 0.3 Gm. of the olive oil, accurately weighed, into a glass-stoppered flask or bottle of 250-cc. capacity, dissolve it in 10 cc. of chloroform, add 25 cc. of iodobromide T.S., accurately measured from a burette or pipette, stopper the vessel securely, and allow it to stand for 30 min.[1] in a cool place protected from light.

Only 0.3 Gm. of olive oil is used in this determination, as its iodine value is comparatively high (79 to 88).[2]

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[1] Allow castor oil, cod liver oil, or linseed oil to stand for 1 hr.

[2] 0.12 to 0.15 Gm. of linseed oil or of cod liver oil, 0.8 to 1.0 Gm. of theobroma oil.

The Pharmacopoeia directs that 0.8 Gm. be taken for solid fats, as their iodine values are low as compared with those of the fatty oils, the iodine value of oil of theobroma, for instance, being 5 to 340.

The oil is soluble in chloroform, and since iodine and iodine bromide are also soluble in this solvent, it serves as a good reaction medium.

The oil, upon standing in contact with the iodobromide test solution, absorbs iodine. Iodine, itself, is absorbed very slowly by the oil, but iodine is absorbed readily from bromine-containing solutions, probably through a reaction of iodine bromide, IBr, wherein the bromine functions as a catalytic agent. The unsaturated acids of the oleic and linoleic series present in the olive oil, as well as their glyceryl esters, absorb iodine to form addition products. Thus oleic acid $C_{17}H_{33}COOH$ takes up 2 atoms of iodine and forms the addition product diiodostearic acid $C_{17}H_{33}I_2COOH$. The mixture, which is directed to be placed in a glass-stoppered flask (Fig. 20) to prevent the escape of iodine and bromine vapors, should also be protected from light to prevent other side reactions from taking place in which iodine is consumed. If after standing for ½ hr. the solution is no longer colored brown, the amount of iodine present was insufficient to saturate the oil.

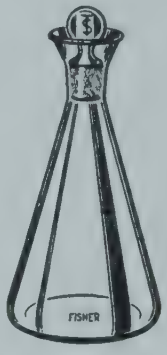


FIG. 20. Iodine titration flask.

2. Then add, in the order named, 30 cc. of potassium iodide T.S. and 100 cc. of water, and titrate the liberated iodine with 0.1*N* sodium thiosulfate, shaking thoroughly after each addition of thiosulfate. When the iodine color becomes quite pale, add 1 cc. of starch T.S. and continue the titration with thiosulfate until the blue color is discharged.

Upon the addition of the potassium iodide solution, the iodine bromide reacts with the KI, liberating iodine until all the bromine is used up:

$$KI + IBr \longrightarrow KBr + I_2$$

An excess of KI is added to ensure the complete removal of the free bromine and to prevent the precipitation of the iodine in the aqueous solution. The sodium thiosulfate reacts with the excess iodine as follows:

$$2Na_2S_2O_3 + I_2 \longrightarrow 2NaI + Na_2S_4O_6$$

3. Carry out a blank test at the same time with the same quantities of the same reagents and in the same manner and titrating as directed. The difference between the number of cubic centimeters of thiosulfate consumed by the blank test and the actual test, multiplied by 1.269 and divided by the weight in Grams of the sample taken, is the Iodine Value.

"NOTE—If more than half of the iodobromide T.S. is absorbed by the portion of the substance taken, the determination must be repeated, a smaller portion of the substance under examination being used."

TABLE 45. OFFICIAL SUBSTANCES WITH THEIR IODINE NUMBERS

Substance	Iodine Number, Official Requirement
U.S.P.	
Almond oil, expressed	95 to 105
Castor oil	83 to 88
Cod liver oil	145 to 180
Cod liver oil, nondestearinated	128 to 180
Corn oil	102 to 128
Cottonseed oil	109 to 116
Halibut liver oil	125 to 155
Halibut liver oil capsules	125 to 155
Oleic acid	85 to 95
Olive oil	79 to 88
Peanut oil	84 to 100
Persic oil	90 to 108
Sesame oil	103 to 116
Soap, soft, medicinal (acids)	(n.l.t.) 85
Sodium morrhuate injection	(n.l.t.) 130
Stearyl alcohol	(n.m.t.) 2
Theobroma oil	35 to 40
Wool fat	18 to 36
Wool fat, hydrous	18 to 36
N.F.	
Cetyl alcohol	(n.m.t.) 5
Chaulmoogra oil	93 to 104
Coconut oil	7 to 10
Glyceryl monostearate	(n.m.t.) 6
Hydroxystearin sulfate	(n.m.t.) 8
Linseed oil	(n.l.t.) 170
Lard	46 to 70
Oleyl alcohol	74 to 80
Soap, hard (acids)	83 to 92
Stearic acid	(n.m.t.) 4
Suet, prepared	33 to 48

(n.m.t.) = not more than. (n.l.t.) = not less than.

The blank test, when carried out under the same conditions as the actual test, corrects for the presence of impurities in the reagents, changes in volume at different temperatures, etc., and makes it unnecessary to know the exact normality of the iodobromide test solution. The blank test should be carried out in a manner exactly duplicating that used in the actual test in so far as possible, especially in the measurement of the iodobromide test solution, which has a high coefficient of expansion. It is required that the determination be repeated if more than half of the iodobromide T.S. is absorbed by the sample to ensure the presence of an excess of iodine.

If in the blank test 25 cc. of 0.1 N $Na_2S_2O_3$ are consumed, and in the actual test 5 cc. are required, the calculations may be made as follows: Since 1 cc. of 0.1 N $Na_2S_2O_3$ is equivalent to $\frac{126.9}{10 \times 1,000} =$ 0.01269 Gm. of iodine, then $25 - 5 = 20$ cc. of 0.1 N $Na_2S_2O_3$, equivalent to the iodine absorbed by the oil, and the weight of iodine that would be absorbed by 100 Gm. of the oil is equal to $\frac{20 \times 0.01269}{0.30} \times 100$ or $\frac{20 \times 1.269}{0.3} = 84.6$, the iodine number of the sample of olive oil.

Questions and Problems

1. Define the term *iodine value*.
2. Indicate by structural formulas how iodine may add on to an unsaturated fatty acid.
3. How may a determination of the iodine number be used to supplement the information relative to the nature and purity of an oil gained through a determination of the saponification value?
4. What functions do the bromine, acetic acid, chloroform, and potassium iodide serve in the determination of the iodine number of an oil?
5. Given an unknown sample of a fatty oil. What observations, tests, and determinations would you perform in order to establish the identity and purity of the oil?

Hydroxyl Value. *The hydroxyl value or hydroxyl number is the number of milligrams of potassium hydroxide equivalent to the hydroxyl content of 1 gram of the substance.* This constant gives an indication of the identity and purity of fatty substances possessing alcoholic hydroxyl groups.

The hydroxyl value is inversely proportional to the molecular weight. Therefore, an abnormally low value is indicative of adulteration with higher molecular weight alcohols or with nonalcoholic fatty substances (paraffin, petroleum oil, etc.).

Exercise 75

Object. To Determine the Hydroxyl Number of Cetyl Alcohol.

Materials Required. 2 Gm. of cetyl alcohol.
2 cc. of pyridine.
10 cc. of toluene.
10 cc. of 1.5*M* acetyl chloride in toluene.
About 30 cc. of 1*N* sodium hydroxide.

Procedure. 1. "Transfer into a dry 250-cc. iodine flask about 2 Gm. of Cetyl Alcohol, accurately weighed, and add exactly 2 cc. of pyridine followed by 10 cc. of toluene, using the latter to rinse the neck of the flask. To the mixture add exactly 10 cc. of approximately 1.5*M* acetyl chloride in toluene, stopper the flask and immerse in a water bath heated to 60° to 65° for 20 minutes."

The determination can be carried out in an iodometric flask or in an ordinary glass-stoppered 250-cc. flask. The approximately 1.5*M* acetyl chloride solution can be conveniently prepared by dissolving 10 cc. of acetyl chloride in 90 cc. of toluene. The toluene serves as a mutual solvent for the solid cetyl alcohol, the acetyl chloride, and the cetyl acetate formed. Pyridine acts as a condensing agent by reacting with the hydrogen chloride formed during the acetylation, which proceeds according to the following equation:

$$CH_3(CH_2)_{14}CH_2OH + CH_3COCl \longrightarrow CH_3(CH_2)_{14}CH_2O_2CCH_3 + HCl$$

At 60 to 65°, the acetylation is complete in a very short time.

2. "Add 25 cc. of distilled water, stopper the flask and shake it vigorously for 1 or 2 minutes and allow to stand for 5 minutes to decompose the excess acetyl chloride. Titrate to a permanent pink end point, shaking the flask vigorously toward the end of the titration to maintain the contents in an emulsified condition, with 1*N* sodium hydroxide, using 1 cc. of phenolphthalein T.S. as the indicator."

The excess acetyl chloride is hydrolyzed to acetic acid by shaking vigorously with water to ensure contact between the water and the acetyl chloride that is dissolved in the oily layer. During the titration of the acetic acid with 1*N* sodium hydroxide, it is again necessary to shake vigorously to ensure complete neutralization of the acetic acid.

3. "Run a blank under identical conditions, using the same amounts of reagents as in the determination. Subtract the number of cubic centimeters of 1*N* sodium hydroxide used for the sample from the number of cubic centimeters used for the blank. Multiply the difference by 56.10 and divide by the weight of sample to obtain the hydroxyl number which is the number of mg. of potassium hydroxide equivalent to the hydroxyl content of 1 Gm. of the sample."

The amount of acetyl chloride that has reacted with the fatty alcohol can be determined from the difference between the amount of 1*N* sodium hydroxide consumed by the excess acetyl chloride in the blank and in the sample. Each cubic centimeter of 1*N* sodium hydroxide is equivalent to 0.0001 mole or 56.1 mg. of potassium hydroxide. Therefore, the expression

$$\frac{\text{cc. of } 1N \text{ NaOH} \times 56.1}{\text{wt. of sample}}$$

gives the hydroxyl number, or the number of milligrams of potassium hydroxide that is equivalent chemically to the acetyl chloride required for the acetylation of the hydroxyl content of 1 Gm. of sample of the fatty alcohol.

The hydroxyl values of official substances are as follows: stearyl alcohol, U.S.P. (200 to 220); cetyl alcohol, N.F. (218 to 238), and oleyl alcohol, N.F. (210 to 230).

Acetyl Value of Fatty Acids. The acetyl value of fatty acids corresponds closely to the hydroxyl value of fatty alcohols, and the two constants have much the same significance with respect to identity and purity of the substances. The acetyl value is found by acetylating hydroxy fatty acids, *e.g.*, hydroxystearic acid or hydrogenated castor oil, and determining the saponification value of the product. From the saponification value of the acetylated fatty acid and the acid value of the original fatty acid, the acetyl value is calculated and expressed as *the number of milligrams of potassium hydroxide required to neutralize the acetic acid obtained by the saponification of 1 gram of acetylated fatty acids.*

The official procedure for the determination of the acetyl value of fatty acids is as follows:

> Boil about 30 Gm. of the fatty acids, obtained as described in the monograph, with 30 cc. of acetic anhydride under a reflux condenser for 2 hr. Pour the mixture into 500 cc. of distilled water contained in an 800-cc. beaker and boil gently for 15 minutes while bubbling carbon dioxide through the mixture. Siphon off the greater part of the water and repeat the boiling operation with 2 successive 500-cc. portions of distilled water. Transfer the acetylated oil into a pear-shaped 500-cc. separator and wash with two 200-cc. portions of distilled water warmed to about 50°. Separate as much of the water as possible and add 5 Gm. of anhydrous sodium sulfate, shake well, allow to stand 30 minutes, then filter through a folded filter, carrying out the filtration in a drying oven at 105°, if necessary.

A 2-Gm. sample of the acetylated fatty acid is then used for the determination of the saponification value (see page 278) and the acid value of the original fatty acid is determined (see page 274). The acetyl value can be calculated using the following formula:

$$A = \frac{S - F}{1 - 0.00075S}$$

where A = acetyl value of the free fatty acids.
S = saponification value of the acetylated fatty acids.
F = acid value of the original fatty acids.

The complete derivation of this formula for calculation of the acetyl value is somewhat involved and the original literature should be consulted.[3] The factor 0.00075 gives the number of grams of acetyl group that corresponds to 1 mg. of potassium hydroxide. The denominator $1 - 0.00075S$ corrects for the discrepancy between the molecular weight of the acetylated oil (used for determination of the saponification value) and that of the original fatty acid (used for determination of the acid value).

The acetyl value of the fatty acids obtained by the hydrolysis of Hydroxystearin Sulfate, N.F. is (n.l.t.) 55 and (n.m.t.) 95.

[3] L. W. Cook, *J. Am. Chem. Soc.*, 44, 392, 1922.

Questions and Problems

1. Define *hydroxyl value* and *acetyl value*. For what types of fatty substances are these constants significant?

2. Give equations for all reactions involved in the determination of the acetyl value of the fatty acids of hydroxystearin sulfate, starting with the hydrolysis of hydroxystearin sulfate.

3. A sample of stearyl alcohol has been adulterated with paraffin wax to the extent that it is 90 per cent pure. Calculate its hydroxyl value.

Water and Sediment in Fatty Oils. Certain fatty oils, particularly those of animal origin, which are not highly refined, frequently contain some moisture and nonfatty tissue residues. Official limits for moisture and sediment have been fixed by the Pharmacopoeia for oils of this type. For example, Nondestearinated Cod Liver Oil must contain not more than 0.5 per cent by volume of water and sediment when treated according to the following directions.

Place exactly 50 cc. of benzene in each of two centrifuge tubes and to each tube add exactly 50 cc. of the oil, warmed if necessary to reincorporate separated stearin, and thoroughly mixed at 25°. Tightly stopper the tubes and shake them vigorously until the contents are thoroughly mixed, then immerse the tubes in a water bath at 50° for 10 minutes. Place the tubes on opposite sides of the centrifuge and whirl for 10 minutes. Read the combined volume of water and sediment at the bottom of each tube. Repeat the whirling for 10-minute periods until the combined volume of water and sediment remains constant for three consecutive readings. The sum of the volumes of combined water and sediment in the two tubes represents the percentage, by volume, of water and sediment in the oil.

The determination is carried out in pear-shaped centrifuge tubes that are graduated in such a way that small volumes can be more accurately read.

Range, cc.	Scale Division, cc.
0 to 3	0.1
3 to 5	0.5
5 to 10	1.0
10 to 25	5.0
25 to 50	25.0
50 to 100	50.0

A centrifuge that has a diameter of swing of 38 to 43 cm. (measured from tip to tip of the whirling tubes), is operated at 1,500 r.p.m. If a centrifuge of different dimensions be used, it should be operated at a rate calculated by using the following formula (where d = diameter in centimeters):

$$\text{r.p.m.} = 1{,}500\sqrt{\frac{40.6}{d}}$$

Chapter 14

ASSAY OF VOLATILE OILS

Volatile oils, also known as *ethereal oils* or *essential oils* and, in some cases, as *essences*, are generally complex products composed of mixtures of compounds of widely variant chemical characteristics. The most important chemical components of the official volatile oils are:

1. *Hydrocarbons*, occasionally of the acyclic series, such as heptane and myrcene, but more often of the isocyclic series, *e.g.*, pinene, camphene, limonene, bornylene, fenchene, dipentene, sylvestrene, and phellandrene.
2. *Alcohols*, present both in the free state and in combination with acids as esters. The alcohols most generally found in the official volatile oils are linalol, geraniol, citronellol, terpineol, borneol, menthol, and santalol.
3. *Aldehydes*, such as benzaldehyde, cinnamic aldehyde, salicyl aldehyde, citral, and citronellal.
4. *Ketones*, the most important being camphor, carvone, fenchone, thujone, and menthone.
5. *Phenols*, such as anethol, eugenol, carvacrol, safrol, chavicol, and thymol.
6. *Acids*, sometimes present in the free state in small quantities, those occurring most commonly being acetic, propionic, butyric, valeric, benzoic, cinnamic, and hydrocyanic acids; but more often occurring in combination with the alcohols mentioned under 2 as esters, *e.g.*, linalyl acetate, bornyl acetate, and menthyl acetate.
7. *Sulfur compounds*, such as the allyl isothiocyanate (mustard oil).

The analysis of volatile oils for the purposes of determining their purity and value is based on the measurement of certain physical characteristics, on the quantitative estimation of certain components as alcohols, phenols, esters, and aldehydes, and on the qualitative tests for the various substances that are commonly employed as adulterants. Only the official quantitative procedures will be discussed here.

Methods of General Application. The purity and quality of volatile oils may be judged to some extent by their appearance, odor, color, etc., but the information gained from the determination of the specific gravity, rotatory power, refractive index, solidifying point, solubility, and behavior on distillation is of much greater importance.

Specific Gravity. The specific gravity of a volatile oil may be determined with the Westphal balance or pycnometer, the latter being the official method and the more accurate of the two, and expressed as the ratio of the weight of the volume of oil to that of an equal volume of pure water when both are determined at 25° (see page 398).

The specific gravities of the official volatile oils vary approximately between 0.84 and 1.2. Those oils which are lighter than water, such as orange, caraway, coriander, lemon, turpentine, and rosemary oils, are usually rich in hydrocarbons, alcohols, esters, and ketones. Oils, the specific gravities of which approach or exceed 1.0, *e.g.*, anise, cinnamon, clove, and sassafras oils, usually contain chiefly aldehydes, phenols, phenolic derivatives, or certain esters.

The specific gravity of any volatile oil is not absolutely constant, since it is influenced by such factors as the maturity of the plant from which the oil is obtained and the age of the oil, as well as by the methods of preparation and purification.

Rotatory Power. The rotatory power of a volatile oil is generally measured with a Laurent half-shadow polarimeter, according to the procedure described on page 487, using sodium light and a tube 10 cm. long, but for highly colored oils, tubes 5 or even 2.5 cm. long may be used. The observation of the optical activities of the official essential oils should be made at 25°. Slight deviations from this temperature do not greatly affect the rotatory power of a volatile oil, except in the cases of lemon oil and orange oil.

The rotatory power of some of the volatile oils varies within relatively wide limits. This determination should never be omitted in their examination, however, since it frequently serves as a valuable means of detecting adulteration with inactive substances, such as alcohol, or with substances of different rotatory power from that of the oil being examined, *e.g.*, lemon oil (+57 to +65.6°) adulterated with turpentine oil (about +25 to −40°).

Refractive Index. The refractive index of a volatile oil is most commonly determined by means of an Abbé refractometer, as described on page 480. The measurement of the refractive indices of the official volatile oils is required to be performed at 20°, except in the case of rose oil, which is measured at 30°. The index of refraction does not vary greatly with different official volatile oils, the values being between about 1.46 and 1.61 at 20°. In some cases, however, this determination may serve for the detection of extraneous matter.

Congealing Temperature or Point. The congealing temperature or point of a volatile oil is determined by the method described on page 422. Most essential oils solidify only at low temperatures. Consequently, in practice, this determination is carried out with only a few oils, such as anise oil, eucalyptus oil, and fennel oil, which contain large amounts of the readily crystallizable constituents anethol and eucalyptol. The higher the congealing temperature of these oils, the more they are valued. An abnormally low congealing temperature of an essential oil indicates the partial removal of the characteristic constituent for which the oil is valued or the addition of extraneous matter, such as alcohol.

Distillation Range or Limits. The distillation range of a volatile oil is determined by the general method described on page 427. Volatile oils that are composed of mixtures of hydrocarbons, alcohols, esters, etc., boil between certain limits of temperature, frequently separated widely. Consequently, the official standards usually designate the temperature or range of temperature at which a definite percentage of the oil distills; *e.g.*, not less than 90 per cent of turpentine oil should distill between 154 and 170°; not less than 95 per cent of pine oil should distill between 200 and 225°, and less than 10 per cent of dwarf pine needle oil should distill below 165°.

Fractional Distillation. Fractional distillation is resorted to occasionally in the official tests for purity of volatile oils. It serves either to separate the various components of volatile oils or to detect adulteration. Thus, alcohol and petroleum ether, both of which boil below 100°, may be separated and identified in this way. The addition of turpentine oil and other adulterants may be detected by collecting various fractions of the oil and determining the rotatory power and refractive index of each fraction; *e.g.*, see Orange Oil, U.S.P., Lemon Oil, U.S.P., and Bitter Orange Oil, N.F.

Solubility. The volatile oils are generally soluble in organic solvents, such as absolute alcohol, ether, chloroform, benzene, carbon disulfide, etc. They dissolve more or less readily in dilute alcohol according to the nature of their components. Oils containing a large percentage of oxygenated substances often produce turbid solutions with petroleum ether or carbon disulfide because of the separation of water, small quantities of which are dissolved in such oils.

Practically all the volatile oils exhibit almost constant solubility in 90, 80, or 70 per cent alcohol. Consequently, the test of the solubility of a volatile oil in dilute alcohol frequently gives valuable data relative to its purity, since the commonly used adulterants, turpentine oil, petroleum oils, and fatty oils are but slightly soluble in 80 or 70 per cent alcohol.

Assay for Ester Content. A number of the official volatile oils are evaluated on the basis of their ester content. The esters found are mostly the acetates of alcohols of the formula $C_{10}H_{17}OH$, *e.g.*, borneol, geraniol, linalol, and terpineol; $C_{10}H_{19}OH$, *e.g.*, menthol or citronellol; or $C_{15}H_{23}OH$, *e.g.*, santalol. When these esters are refluxed with alcoholic potassium hydroxide, they are saponified and yield the free alcohol and the potassium salt corresponding to the acid component of the ester.

The number of milligrams of KOH required to saponify 1 Gm. of the oil represents the *ester value* or *ester number* of the oil. The ester value for any given oil may be subject to considerable variation, but the minimum requirement set by the official standards must be complied with when the oil is represented as a product conforming to the U.S.P. or N.F. standards. The determination of the total esters when taken in conjunction with the official tests for purity serves to detect adulteration and to establish the quality of those oils valued for their ester content.

The ester values of oils that contain appreciable amounts of aldehydes or phenols cannot be estimated accurately by saponification with an alkali, since variable amounts of the latter are consumed by reaction with the aldehydes and phenols, or their decompositon products.

Exercise 76

Object. To Prepare 0.5N Alcoholic Potassium Hydroxide.

Materials Required. 2.5 Gm. of lead acetate.
1,500 cc. of alcohol.
40 Gm. of potassium hydroxide.

Procedure. 1. Dissolve 2.5 Gm. of lead acetate in 5 cc. of water, add the solution to 1,000 cc. of alcohol contained in a glass-stoppered bottle, and mix thoroughly. Dissolve 5 Gm. of potassium hydroxide in 25 cc. of warm alcohol, cool the solution, and add it slowly, without stirring, to the alcohol solution of lead acetate. After 1 hr. shake the mixture vigorously, allow it to stand overnight, decant the clear liquid, and recover the alcohol by distillation.

The potassium hydroxide converts any acids present into nonvolatile salts and causes the polymerization of aldehydes. The lead acetate is decomposed by the KOH with the formation of lead oxide and potassium acetate. The lead forms insoluble and nonvolatile compounds with any sulfur compounds present. The lead oxide carries down the polymerized aldehydes when it is precipitated so that it is easy to decant the clear supernatant liquid.

2. Dissolve about 35 Gm. of potassium hydroxide in 20 cc. of water and add sufficient aldehyde-free alcohol to make 1000 cc. Allow the solution to stand in a tightly-stoppered bottle (using either a glass or a rubber stopper) for 24 hours. Then quickly decant the clear supernatant liquid into a bottle provided with a well-fitting rubber stopper and standardize as follows:

Traces of aldehydes remaining in the alcoholic potassium hydroxide solution are polymerized and settle out when the mixture is allowed to stand. The rubber stopper used should be washed with a small quantity of the alkaline alcoholic solution before it is inserted into the mouth of the bottle, to dissolve sulfur and any other material that might affect the concentration of the solution.

3. Measure accurately, from a burette, about 25 cc. of 0.5*N* hydrochloric acid. Dilute with 50 cc. of water, add 2 drops of phenolphthalein T.S., and titrate with the alcoholic potassium hydroxide solution until a permanent, pale pink color is produced. Calculate the normality.

The titration is carried out as described on page 101. The prepared solution should be preserved in tightly stoppered bottles to prevent changes in the concentration of alkali through absorption of CO_2. Well-fitting rubber stoppers may be used. It should also be protected from light to prevent the development of reddish-colored compounds, which interfere with the correct determination of the end point during titration. The solution should be standardized whenever it is used, and it should always be measured at the same temperature at which it was standardized, since alcohol has a high coefficient of expansion.

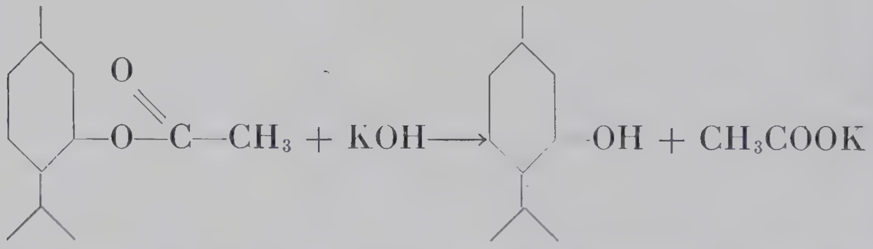
Exercise 77

Object. Assay of Peppermint Oil for Total Esters.

Materials Required. 10 cc. of peppermint oil.
25 cc. of 0.5*N* alcoholic KOH.
About 25 cc. of 0.5*N* H_2SO_4.

Procedure. 1. "Place 10 cc. of Peppermint Oil in a tared, 125-cc. Erlenmeyer flask, and weigh it accurately. Add 25 cc. of 0.5*N* alcoholic potassium hydroxide, connect the flask with a reflux condenser, and boil the mixture on a water bath for exactly 1 hour."

Instead of weighing the oil directly in a previously tared Erlenmeyer flask, it may be weighed in a weighing bottle, and the weighing bottle with its contents may then be transferred into the Erlenmeyer flask. The esters of peppermint oil, which consist chiefly of menthyl acetate, are saponified by the alkali when the mixture is refluxed, forming free menthol and potassium acetate. Alcoholic potassium hydroxide is used because the oil is soluble in strong alcoholic solution.



2. "Allow the mixture to cool, and titrate the excess alkali with 0.5*N* sulfuric acid, using 10 drops of phenolphthalein T.S. as the indicator.

The number of cubic centimeters of 0.5N alcoholic potassium hydroxide consumed in the saponification, multiplied by 0.09915, represents the number of grams of esters, calculated as menthyl acetate, in the oil taken for the assay."

The 0.5N acid neutralizes the excess 0.5N alkali:

$$2KOH + H_2SO_4 \longrightarrow K_2SO_4 + 2H_2O$$

The difference between the number of cubic centimeters of 0.5N KOH added and the number of cubic centimeters of 0.5N H_2SO_4 required in the titration represents the alkali consumed in the saponification of the esters present in the oil; *e.g.*, if a 9-Gm. sample of the oil refluxed with 25 cc. of 0.5N KOH required 20.5 cc. of 0.5N H_2SO_4 to neutralize the excess alkali, the amount of standard alkali solution consumed in the saponification of the esters present in the oil would be 25 − 20.5 = 4.5 cc. The percentage of total esters present in the oil calculated as menthyl acetate is found as follows: $\frac{4.5 \times 9.915}{9}$ = 4.96 per cent of menthyl acetate. The factor 9.915 is derived from the molecular weight of menthyl acetate, 198.30. Thus, 1,000 cc. of N KOH is equivalent to 198.30 Gm. of menthyl acetate, and 1 cc. of 0.5N KOH is equivalent to $\frac{198.24}{2 \times 1{,}000}$ =

TABLE 46. OFFICIAL VOLATILE OILS ASSAYED FOR THEIR ESTER CONTENT

Oil	Amount used, cc.	Equivalent of 1 cc. of 0.5N KOH, Gm.	Official requirement, per cent
U.S.P.			
Lavender oil	5	0.09814	Linalyl acetate $C_{10}H_{17}C_2H_3O_2$ = 30
Methyl salicylate	2	0.07670	Methyl salicylate $C_6H_4(OH)CO_2CH_3$ = 98
Peppermint oil	10	0.09915	Menthyl acetate $C_{10}H_{19}C_2H_3O_2$ = 5
Rosemary oil	10	0.09814	Bornyl acetate $C_{10}H_{17}C_2H_3O_2$ = (n.l.t.) 1.5
N.F.			
Bergamot oil	2*	0.09814	Linalyl acetate $C_{10}H_{17}C_2H_3O_2$ = (n.l.t.) 36
Pine needle oil, dwarf	10	0.09814	Bornyl acetate $C_{10}H_{17}C_2H_3O_2$ = 3 to 10

* Gm.
(n.l.t.) = not less than.

0.09915 Gm. of menthyl acetate. This equivalent is multiplied by 100 so that the factor 9.915 when multiplied by the number of cubic centimeters of $0.5N$ alkali consumed in the assay and divided by the weight of the sample of oil used will give the percentage of esters directly.

Questions and Problems

1. What effect would the presence of free acids in the oil have upon the result of the determination of total esters?

2. Why is an alcoholic rather than an aqueous solution of KOH used to saponify the esters?

3. What factor would be necessary for the ester in order to obtain the per cent of total esters directly as described above, if the chief ester present in an oil was bornyl acetate; linalyl acetate? Show how these factors are derived.

4. Note the equivalents given in Table 46 for linalyl acetate in lavender oil, U.S.P., and in bergamot oil, N.F. Which is correct?

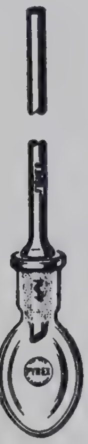


FIG. 21. Acetylization flask with air condenser.

Assay for Alcohol Content. The alcohols present in volatile oils, such as menthol and borneol, occur both free and combined as esters. Usually, the determination of the total alcohol, occurring free and combined, is sufficient to establish the purity and value of an oil with respect to its content of alcoholic constituents.

The total alcohols present in any given oil are determined by transforming the free alcohols into the corresponding acetates by boiling the oil with acetic anhydride in an acetylization flask (Fig. 21) and then determining the saponification value of the acetylized product. When the alcohol occurs partly free and partly combined in the form of an ester, a correction factor must be employed. The method of determining the total alcohol content of volatile oils is illustrated in the following exercise.

Exercise 78

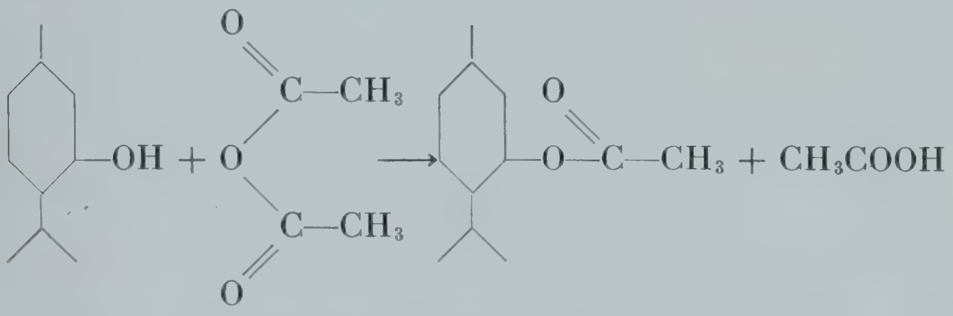
Object. Assay of Peppermint Oil for Total Menthol.

Materials Required. 10 cc. of peppermint oil.
10 cc. of acetic anhydride.

1 Gm. of anhydrous sodium acetate.
3 Gm. of monohydrated sodium carbonate.
About 0.2 Gm. of fused calcium chloride.
50 cc. of 0.5*N* alcoholic KOH.
50 cc. of 0.5*N* H_2SO_4.
An acetylization flask of about 100-cc. capacity with an air condenser about 100 cm. long.

Procedure. 1. "Place 10 cc. of Peppermint Oil in an acetylization flask of 100-cc. capacity, and add 10 cc. of acetic anhydride and 1 Gm. of anhydrous sodium acetate. Boil the mixture gently for exactly one hour, cool, disconnect the flask from the condenser, transfer the mixture to a small separator, rinsing the acetylization flask with three successive 5-cc. portions of warm water, and add the rinsings to the separator."

The acetylization flask should be supported on an asbestos board while heating to prevent decomposition of the oil. The acetic anhydride reacts with the menthol, forming menthyl acetate and acetic acid. The acetylization is promoted by the anhydrous sodium acetate.



2. "When the liquids have completely separated, reject the water layer, and wash the remaining oil with successive portions of sodium carbonate T.S., diluted with an equal volume of water, until the last washing is alkaline to 2 drops of phenolphthalein T.S. Dry the resulting oil with anhydrous sodium sulfate and filter."

The oil is washed with sodium carbonate test solution (12.5 Gm. of $Na_2CO_3.H_2O$ in sufficient distilled water to make 100 cc.) to neutralize the excess acetic acid. The washed oil is then dried over anhydrous sodium sulfate to remove the small amounts of alkaline sodium carbonate solution that it contains.

3. "Transfer 5 cc. of the dry acetylized oil to a tared, 100-cc. Erlenmeyer flask, note its exact weight, add 50 cc. of 0.5*N* alcoholic potassium hydroxide,

connect the flask with a reflux condenser, and boil the mixture on a water bath for exactly one hour."

The $0.5N$ KOH saponifies the menthyl acetate, forming menthol and potassium acetate in the same manner as in the assay for total esters. The liberated menthol dissolves in the alcoholic solution.

4. "Allow the mixture to cool, and titrate the excess of alkali with $0.5N$ sulfuric acid, using 10 drops of phenolphthalein T.S. as the indicator. Calculate the per cent of menthol by the following formula:

Per cent of total menthol in the Oil tested

$$= \frac{A \times 7.813}{B - (A \times 0.021)} \times [1 - (E \times 0.0021)]$$

"A is the result obtained by subtracting the number of cubic centimeters of $0.5N$ sulfuric acid required in the above titration from the number of cubic centimeters of $0.5N$ alcoholic potassium hydroxide originally taken. B is the weight of acetylized oil taken, and E is the percentage of esters calculated as menthyl acetate ($C_{10}H_{19}.C_2H_3O_2$)."

The number of cubic centimeters of $0.5N$ KOH consumed in the saponification of a definite weight of the acetylized oil is equal to the difference between the number of cubic centimeters of $0.5N$ KOH added and the number of cubic centimeters of $0.5N$ H_2SO_4 required in the residual titration. The formula used in the calculation is derived as follows: The molecular weight of menthol is 156.26. One cubic centimeter of $0.5N$ KOH is equivalent to $\frac{156.26}{2 \times 1{,}000} = 0.07813$ Gm. of menthol. In order that the result may be read directly in terms of per cent, the factor 0.07813 is multiplied by 100, giving 7.813. $B - (A \times 0.021)$ corrects for the weight of acetyl radical in the sample of acetylized oil, the factor 0.021 being the amount of acetyl radical equivalent to 1 cc. of $0.5N$ KOH. The latter is obtained from 198.30 (molecular weight of menthyl acetate) $-$ 156.26 (molecular weight of menthol) $=$ 42.04 (molecular weight of the acetyl radical). Therefore, 1,000 cc. of N KOH is equivalent to 42.04 Gm. of acetyl radical, and 1 cc. of $0.5N$ KOH is equivalent to $\frac{42.04}{2 \times 1{,}000} = 0.021$ Gm. of acetyl radical.

$(A \times 0.021)$, therefore, represents the weight of acetyl radical to be deducted from B, the weight of acetylized oil taken.

Since the original oil contained some menthol combined as ester and calculated as menthyl acetate, it would be incorrect to subtract all the weight of the acetyl radical from B. A correction for the weight of acetyl radical in the original oil is consequently applied by multiplying the per cent found from the formula $\dfrac{A \times 7.813}{B - (A \times 0.021)}$ by the correction formula $[1 - (E \times 0.0021)]$. The factor 0.0021 is the ratio of the weight of acetyl radical to that of menthyl acetate $42.04/198.30 = 0.21$. If E, the per cent of total esters determined by the preceding assay, is found to be 5, then the formula will be $[1 - (0.05 \times 0.21)]$ or $[1 - (5 \times 0.0021)]$. Applying this correction, the formula becomes $\dfrac{A \times 7.813}{B - (A \times 0.021)} \times [1 - (5 \times 0.0021)]$ = per cent of total menthol.

The importance of the correction $[1 - (0.05 \times 0.21)]$ may be seen from the following example: A sample of peppermint oil found to contain 5 per cent of total esters, calculated as menthyl acetate, and 50 per cent of total menthol, as calculated from the formula, per cent of total menthol $= \dfrac{A \times 7.813}{B - (A \times 0.021)}$, gave the following percentage of total menthol when the correction was applied: $50 \times [1 - (5 \times 0.0021)] = 50 \times (1 - 0.0105) = 50 \times 0.9895 = 49.475$ per cent of menthol free and as esters. The correction involves about one-half of 1 per cent menthol or $50 - 49.475 = 0.525$ per cent.

A similar correction is applied in the assay of rosemary oil, but none is given in the assay of santal oil, since the santalol does not occur combined in the form of esters.

Questions and Problems

1. Having determined the ester value and the percentage of total borneol present in rosemary oil, how could the percentage of free borneol be found? Give an example.

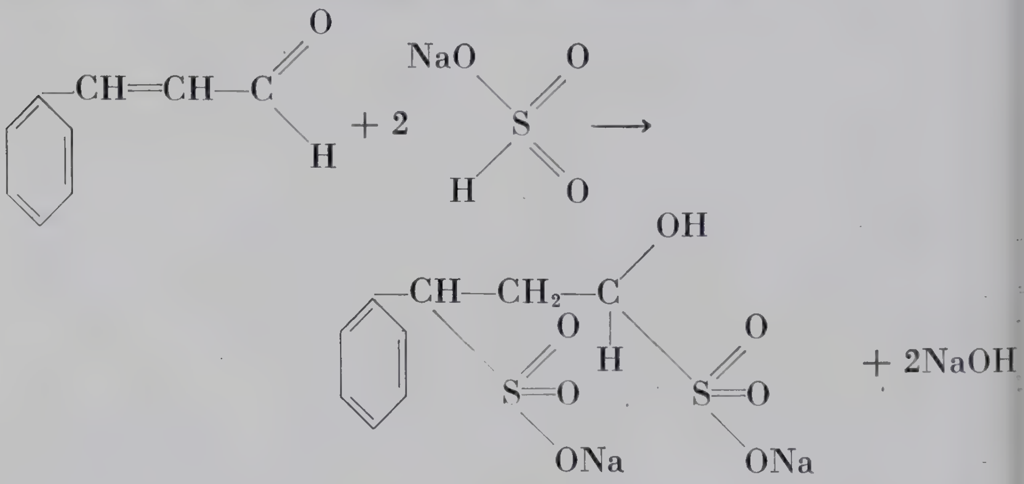
2. Write the reactions that take place in the assay of oil of rosemary for total borneol.

3. If peppermint oil containing 7.2 per cent of total esters is found to contain 51.5 per cent of total menthol (uncorrected), what change in the result would the correction 1 − (*E* × 0.0021) cause? Is this correction important?

TABLE 47. OFFICIAL VOLATILE OILS ASSAYED FOR THEIR ALCOHOL CONTENT

Oil	Amount of acetylized oil used, Gm.	Equivalent of 1 cc. of 0.5*N* KOH, Gm.	Correction factor	Official requirement, per cent
U.S.P.				
Peppermint oil........	5	0.07813	1 − (E × 0.0021)	Menthol $C_{10}H_{19}OH$ = 50
Rosemary oil..........	5	0.07712	1 − (E × 0.0021)	Borneol $C_{10}H_{17}OH$ = 8
N.F.				
Santal oil.............	5	0.11017	None	Santalol $C_{15}H_{23}OH$ = 90

Assay for Aldehyde Content. Aldehydes, like ketones, form addition products with certain reagents, and this property is utilized in their assay. When no other constituents are present which react with the reagent, the assay for aldehydes may be performed by the sulfite method described under the assay for ketones (page 309). Thus, the cinnamic aldehyde content of the official cinnamon oil is determined by this method, the basic reaction being



The bisulfite addition product dissolves in water, leaving the nonaldehyde constituents as a water-insoluble layer. The volume of this water-insoluble layer is then measured in a cassia flask (Fig. 22).

The cassia flask is a glass flask of about 100-cc. capacity with a long, narrow neck graduated to 10 cc. in 0.1-cc. divisions. In the assay for aldehydes and ketones by the sulfite method or bisulfite method, the portion of the oil that does not react to form a water-soluble addition product rises to the surface, and when the flask is filled, this oily layer rises into the graduated neck of the cassia flask, where its volume can be measured. The volume of the residual oily layer subtracted from the volume of the sample of oil used represents the volume of the aldehyde constituents that formed a water-soluble addition product with the sulfite.

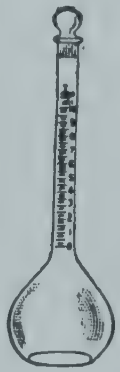


Fig. 22. Cassia flask.

Those volatile oils which contain aldehydes in very small amounts, such as lemon oil, or which contain other constituents that form water-soluble addition products with sodium sulfite and bisulfite cannot be assayed accurately by this method. In these cases, the aldehydes are generally determined by the hydroxylamine method described in the following exercise.

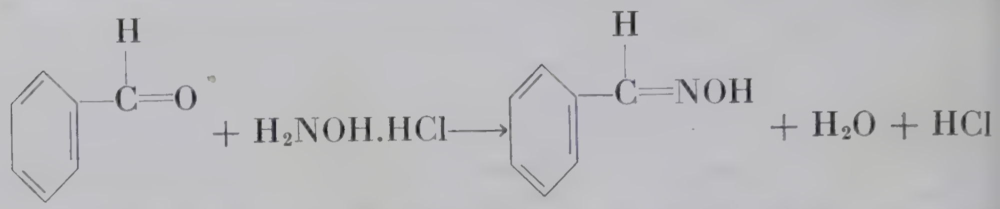
Exercise 79

Object. Assay of Bitter Almond Oil for Benzaldehyde.

Materials Required. 1 cc. of bitter almond oil.
100 cc. of hydroxylamine hydrochloride solution.
Bromophenol blue T.S.
1*N* NaOH.

Procedure. 1. Transfer about 1 cc. of Bitter Almond Oil to a tared glass-stoppered weighing bottle and weigh accurately. Loosen the stopper and transfer the weighing bottle and contents to a 250-cc. Erlenmeyer flask containing 25 cc. of a reagent prepared by dissolving 34.7 Gm. of hydroxylamine hydrochloride in 160 cc. of water and diluting this solution to 1,000 cc. with alcohol. Rinse down the sides of the flask with an additional 50 cc. of the reagent.

The hydroxylamine hydrochloride reacts with the benzaldehyde in the oil to form benzaldoxime, liberating hydrochloric acid.



A standing period of at least 10 min. is necessary for completion of this reaction. A somewhat longer period might, in fact, be desirable, if results are consistently too low.

2. Allow to stand for 10 min., add 1 cc. of bromophenol blue T.S., and titrate the liberated hydrochloric acid to a light green end point with 1*N* sodium hydroxide. Run a blank using the same quantities of reagents but omitting the Bitter Almond Oil. Match the color of this end point in the above assay. To determine the required number of cubic centimeters of 1*N* sodium hydroxide to neutralize the liberated hydrochloric acid, subtract the number of cubic centimeters of 1*N* sodium hydroxide used in the blank from the number of cubic centimeters required in the assay. Each cubic centimeter of 1*N* sodium hydroxide is equivalent to 106.1 mg. of C_7H_6O.

Since benzaldehyde reacts mole for mole with hydroxylamine, each cubic centimeter of 1*N* NaOH used by the sample but not by the blank, *i.e.*, the difference, is equivalent to 0.001 mole of benzaldehyde or to 0.1061 Gm.

The HCN present in the bitter almond oil does not influence the accuracy of the assay. Hydrocyanic acid is a very weak acid. In the presence of a strong acid, such as HCl, the common hydrogen ion represses the dissociation of the weak acid, HCN, so that at the end point, which, in this case, is at a pH of about 3.45, none of the HCN enters into the reaction.

TABLE 48. OFFICIAL VOLATILE OILS ASSAYED FOR ALDEHYDE CONTENT

Oil	Amount used	Method	Official requirement, per cent
U.S.P.			
Cinnamon oil . . .	10 cc.	Bisulfite	Cinnamic aldehyde = (n.l.t.) 80
N.F.			
Almond oil, bitter	1 Gm.	Hydroxylamine	Benzaldehyde = (n.l.t.) 80
Benzaldehyde . .	1 Gm.	Hydroxylamine	Benzaldehyde = (n.l.t.) 98

(n.l.t.) = not less than.

Questions and Problems

1. Show how the equivalent factor for benzaldehyde is derived.
2. Why is the titration carried out in a hydroalcoholic solution?
3. Look up the method of assay for benzaldehyde in the National Formulary and write equations for the reactions that take place.
4. Does the presence of HCN cause an appreciable error in the assay of bitter almond oil for benzaldehyde content?

Assay for Ketone Content. Only three of the official volatile oils are assayed for their ketone content. The determination is made by the so-called "sulfite method" or by the hydroxylamine method. The former method is based on the reaction of ketones with sodium bisulfite to form water-soluble addition compounds.

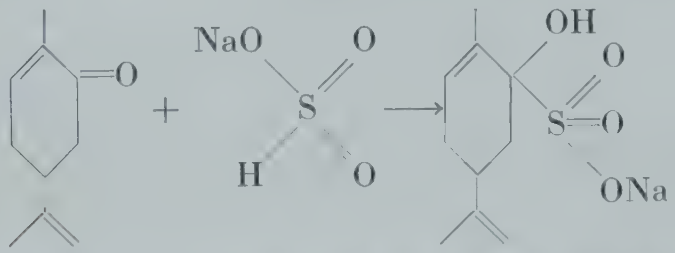
Exercise 80

Object. Assay of Caraway Oil.

Materials Required. A 100-cc. cassia flask.
10 cc. of caraway oil.
100 cc. of saturated sodium sulfite solution (40 Gm. in 100 cc.).
100 cc. of sodium bisulfite solution (5 per cent).

Procedure. 1. "Introduce 10 cc. of Caraway Oil, accurately measured, into a cassia flask, and add 50 cc. of a saturated solution of sodium sulfite which has been carefully rendered neutral to 2 drops of phenolphthalein T.S. by means of a saturated sodium bisulfite solution. Heat the flask in boiling water and shake it repeatedly, neutralizing the mixture from time to time by the addition of a few drops of saturated sodium bisulfite solution."

Carvone, the principal ketone present in the oil, reacts with the sodium bisulfite, forming a water-soluble addition product as follows:



The sodium sulfite is hydrolyzed in part by the water with the formation of sodium hydroxide, *viz.:*

$$Na_2SO_3 + H_2O \longrightarrow NaHSO_3 + NaOH$$

The presence of free alkali in the aqueous layer renders other components of the oil, such as phenols, water-soluble and also tends to reverse the addition reaction. To prevent this the sodium hydroxide is neutralized by the addition of sodium acid sulfite:

$$NaHSO_3 + NaOH \longrightarrow Na_2SO_3 + H_2O$$

2. "When no coloration appears upon adding a few more drops of phenolphthalein T.S. and heating for 15 minutes, cool the mixture to room temperature, and when the liquids have separated completely, add sufficient sodium sulfite solution to raise the lower limit of the oily layer within the graduated portion of the neck of the flask. Note the volume of the residual oily liquid. This volume does not exceed 5 cc., indicating the presence in caraway oil of not less than 50 per cent, by volume, of carvone ($C_{10}H_{14}O$)."

The difference between the volume of oil used as the sample and the volume of the oily layer that remains insoluble represents the carvone which dissolved in the aqueous layer. If drops of oil adhere to the walls of the flask, they may be made to rise into the neck by gently tapping and rotating it. If the residual liquid measures 4.5 cc., the percentage of carvone by volume in the oil would be $\frac{10 - 4.5}{10} \times 100 = 55$ per cent.

Another official oil evaluated for its ketone content is spearmint oil. It is assayed in exactly the same way as is caraway oil, and it is required to contain not less than 50 per cent by volume of carvone. Cedar leaf oil is required to contain not less than 60 per cent of ketones, calculated as thujone ($C_{10}H_{16}O$) when assayed by a hydroxylamine method similar in principle to that previously described for bitter almond oil.

Questions and Problems

1. Ketones may also be estimated in volatile oils by the use of sodium bisulfite alone. Write the reaction between carvone and sodium bisulfite.
2. What substances, if present in the oil, would cause an error in the results of the sulfite method of assay for ketones?
3. How could the carvone be isolated from the aqueous solution?

Assay for Phenol Content. Volatile oils that contain phenols when shaken with solutions of sodium or potassium hydroxide diminish in volume because of the ready solubility of the phenol constituents in alkali; the nonphenolic portion of the oil remains undissolved.

Use is made of this property of phenols in the official assay methods. In these determinations a flask with a graduated neck, like that employed in the estimation of aldehydes (see page 307), is used. In this flask, an accurately measured volume of the oil is shaken vigorously with a portion of the caustic alkali, and a further quantity of alkali is then added to bring the undissolved oil into the graduated neck of the flask. When the liquids have separated and become clear, the volume of the oil insoluble in alkali is read off, the volume of the phenol being determined by difference.

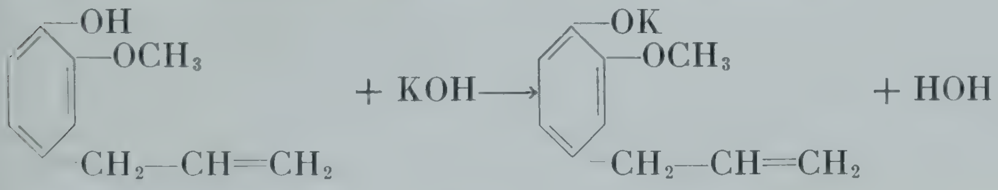
Exercise 81

Object. Assay of Clove Oil.

Materials Required. 10 cc. of clove oil.
100 cc. of normal KOH.
A cassia flask.

Procedure. 1. "Place 10 cc. of Clove Oil, measured from a pipette, in a 100-cc. cassia flask, add 75 cc. of 1*N* potassium hydroxide solution, shake the mixture for 5 minutes, and heat it for 10 minutes in a bath containing boiling water. Remove it from the bath, and cool to room temperature."

The KOH reacts with the phenol, eugenol, as follows:



The mixture is heated on a water bath for 10 min. to saponify any eugenyl acetate present.

2. "When the liquids have separated completely, add sufficient 1*N* potassium hydroxide to raise the lower limit of the oily layer within the graduated portion of the neck: the volume of the oily layer should not exceed 1.5 cc., indicating the presence in the oil of not less than 85 per cent, by volume, of eugenol ($C_{10}H_{12}O_2$)."

The potassium eugenolate dissolves in the water leaving undissolved hydrocarbons, ketones, etc., which rise into the graduated neck of the flask and are measured. If stronger solutions are employed, the results obtained are too high, since the strong alkali exercises some solvent action on the nonphenolic constituents, particularly on oxygenated compounds. The volume of the undissolved liquid subtracted from the volume of oil used as the sample gives the volume of eugenol that dissolved in the alkali; *e.g.*, if a 10-cc. sample of oil yields 1.6 cc. of residual liquid, then $10 - 1.6 = 8.4$ cc. of eugenol dissolved, and the percentage of eugenol in the sample is $\frac{8.4}{10} \times 100 = 84$ per cent.

TABLE 49. OFFICIAL VOLATILE OILS ASSAYED FOR PHENOL CONTENT

Oil	Amount used, cc.	Alkali used	Official requirement, per cent
U.S.P.			
Clove oil.	10	KOH	Eugenol $C_{10}H_{12}O_2$ = (n.l.t.) 82
N.F.			
Myrcia oil.	10	KOH	Phenols = 50 to 65
Pimenta oil.	10	KOH	Phenols = (n.l.t.) 65
Thyme oil.	10	KOH	Phenols = (n.l.t.) 40

(n.l.t.) = not less than.

Questions and Problems

1. Is the assay of phenols as given above an exact method?
2. How could the eugenol be liberated from the alkaline solution?
3. If a part of the eugenol is present combined as esters, is that part estimated in the determination?

Assay for Hydrocyanic Acid Content. Only one official volatile oil is assayed for its hydrocyanic acid content, *viz.*, bitter almond oil.

Exercise 82

Object. Assay of Bitter Almond Oil for Hydrocyanic Acid.

Materials Required. 0.75 Gm. of magnesium sulfate.
0.5*N* sodium hydroxide.
Potassium chromate T.S. (10 Gm. in 100 cc. of water).
0.1*N* silver nitrate.

Procedure. 1. "Dissolve 750 mg. of magnesium sulfate in 45 cc. of distilled water, add 5 cc. of 0.5*N* sodium hydroxide and 2 drops of potassium chromate T.S., and titrate the solution with 0.1*N* silver nitrate to the production of a permanent reddish color."

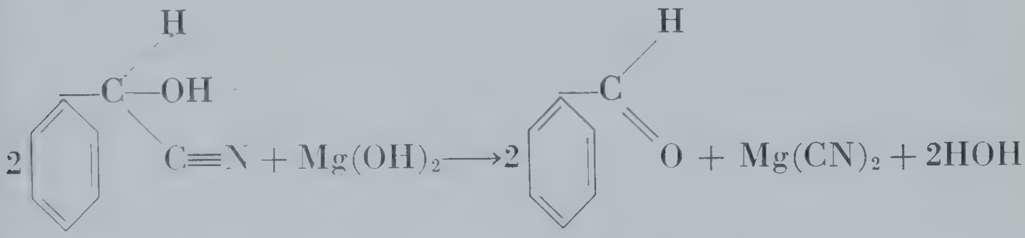
The magnesium sulfate reacts with the sodium hydroxide, forming magnesium hydroxide and sodium sulfate:

$$MgSO_4 + 2NaOH \longrightarrow Mg(OH)_2 + Na_2SO_4$$

The resulting mixture is titrated with 0.1*N* silver nitrate to the appearance of a permanent end point to correct for the presence of impurities in the reagents used, which would react with the silver nitrate in the next step of the assay.

2. "Pour this mixture into a 100-cc. flask containing 1 Gm. of Bitter Almond Oil, accurately weighed, mix well, and titrate again with 0.1*N* silver nitrate until a red color, which does not disappear on shaking, is produced. Conduct this titration as rapidly as possible. Each cubic centimeter of 0.1*N* silver nitrate is equivalent to 2.703 mg. of HCN."

The freshly precipitated $Mg(OH)_2$ splits up the cyanhydrin present in the oil and at the same time furnishes a white background against which the end point of titration may be observed. The reaction may be represented as follows:



Upon titration with 0.1*N* silver nitrate, silver cyanide is formed:

$$Mg(CN)_2 + 2AgNO_3 \longrightarrow 2AgCN + Mg(NO_3)_2$$

When all the alkali cyanide present in the mixture is consumed in the reaction with the silver nitrate, the end point is shown by the formation of the red-colored precipitate of silver chromate:

$$K_2CrO_4 + 2AgNO_3 \longrightarrow Ag_2CrO_4 + 2KNO_3$$

The flask should be shaken continuously during the titration to bring the oil into intimate contact with the mixture. The titration is directed to be conducted as rapidly as possible, because HCN decomposes in the presence of water and alkalies, with the formation of ammonia, formic acid, etc.

Each cubic centimeter of 0.1*N* $AgNO_3$ consumed in the final titration is equivalent to $\frac{27.03}{10 \times 1{,}000} = 0.002703$ Gm. of HCN where 27.03 is the molecular weight of hydrocyanic acid.

Questions and Problems

1. Why is the magnesium sulfate added?
2. From the quantities of $MgSO_4$ and NaOH used, calculate whether any NaOH as such will be present in the reaction mixture.
3. Indicate by equations how HCN may be hydrolyzed in the presence of alkalies with the formation of formates and ammonia.

Assay of Chenopodium Oil for Ascaridol Content. The principal constituent of chenopodium oil from a pharmaceutical standpoint is ascaridol, an organic perioxide, which is valued for its anthelminthic properties. Various chemical and biological methods of evaluating the oil have been proposed, but the official method, which is based on the solubility of ascaridol in acetic acid, is probably as good as any, although it should be regarded as approximate only.

Exercise 83

Object. Assay of Chenopodium Oil.

Materials Required. 2.5 Gm. of chenopodium oil.

50 cc. of 90 per cent acetic acid.

3 cc. of potassium iodide solution (8.3 Gm. of KI dissolved in sufficient distilled water to make 10 cc.).

5 cc. of hydrochloric acid.

10 cc. of glacial acetic acid.

0.1*N* sodium thiosulfate.

One 50-cc. volumetric flask.

One burette graduated in twentieths of a cubic centimeter.

One glass-stoppered tube, 150 mm. in length and 25 mm. in diameter.

Three graduated 10-cc. pipettes.

Procedure. 1. "Place about 2.5 Gm. of Chenopodium Oil, accurately weighed, in a 50-cc. volumetric flask, fill to the mark with 90 per cent acetic acid, mix well, and transfer a portion of this freshly prepared solution to a burette, graduated in 20ths of a cubic centimeter."

The ascaridol in the oil dissolves in the 90 per cent acetic acid. Any variation in the strength of the acetic acid employed causes variable amounts of the constituents of the oil (decomposition and reduction products of ascaridol) to dissolve in the acetic acid; consequently, the acetic acid solution used must be of exactly 90 per cent strength.

2. "Into a glass-stoppered tube, about 150 mm. in length and 25 mm. in diameter, measure, from graduated pipettes, 3 cc. of a solution of potassium iodide (prepared by dissolving 8.3 Gm. of potassium iodide in sufficient distilled water to make 10 cc. of solution), 5 cc. of hydrochloric acid, and 10 cc. of glacial acetic acid. Immerse the tube in a freezing mixture until the temperature is reduced to −3°."

When the acetic acid solution of the oil is added to the reaction mixture, iodine is liberated as a result of the interaction of the ascaridol, which is an organic peroxide, the potassium iodide, and the hydrochloric acid. At usual room temperatures, the reaction is rapid and vigorous, and considerable heat is generated. To delay the reaction and hold the temperature down, and thus guard against the loss of iodine through volatilization, glacial acetic acid is added, and the reagent mixture is chilled before adding the solution of oil.

3. "Add about 5 cc. of the acetic acid solution of the Oil, mix it with the cooled reagent as rapidly as possible, and observe the volume withdrawn from the burette after 2 minutes, to allow for draining. Set the stoppered tube aside at a temperature between 5° and 10°, for exactly 5 minutes."

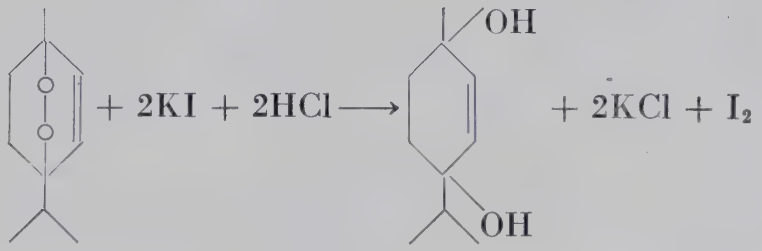
The mixture of reagent and solution of oil is set aside for 5 min. before titrating to make sure that sufficient time has elapsed for the reaction between the ascaridol and the reagent to reach completion. During this time, the temperature is kept down between 5 and 10° for the reasons stated above.

4. "Then, without diluting, titrate the liberated iodine with 0.1*N* sodium thiosulfate. At the same time, conduct a blank test, but dilute the reagent with 20 cc. of distilled water before titrating the liberated iodine. The difference between the two titrations represents the iodine liberated by ascaridol. Each cubic centimeter of 0.1*N* sodium thiosulfate is equivalent to 6.65 mg. of ascaridol $C_{10}H_{16}O_2$."

The directions given in the National Formulary for titration should be closely followed. Dilution of the mixture before titration will vitiate the results.

Some iodine is liberated in the reagent mixture because of dissolved atmospheric oxygen. It is therefore necessary to run a blank assay and to take the difference between the two titrations for calculating the number of cubic centimeters of 0.1*N* sodium thiosulfate consumed.

The reaction of the constituents of chenopodium oil with potassium iodide in acid medium cannot be expressed by a simple stoichiometric equation for either or both of two reasons: (1) there are a number of substances in chenopodium oil that oxidize potassium iodide or react with iodine; (2) the reaction of ascaridol with potassium iodide is more complex than the ordinary reaction of simple organic peroxides with potassium iodide. For example, if the following reaction alone were involved, then 1 cc. of 0.1*N* sodium thiosulfate would represent 8.41 mg. of ascaridol, $C_{10}H_{16}O_2$.



The equivalence of 1 cc. of 0.1*N* sodium thiosulfate and 6.65 mg. of ascaridol, as given in the National Formulary, is empirical and is predicated on the close adherence to the prescribed conditions.

Questions and Problems

1. Look up the reaction of organic peroxides with acids in a textbook on organic chemistry and note whether the probable reaction indicated conforms to the information obtained.

2. Calculate the theoretical ascaridol equivalent of 1 cc. of 0.1*N* $Na_2S_2O_3$ assuming that a molecule of ascaridol liberates 2 atoms of iodine.

Assay for Allyl Isothiocyanate. Black mustard oil is assayed for its content of allyl isothiocyanate. The volatile oil is separated by distillation and its allyl isothiocyanate content determined.

Determination of Volatile Oil Content of Crude Drugs and Oleoresins. Certain crude drugs and oleoresins, used as medicinal or flavoring agents, owe their virtues primarily to volatile oil constituents. Official assay methods for drugs and preparations of these types have been developed, and the limits for volatile oil content have been fixed in a number of instances as shown in Table 50.

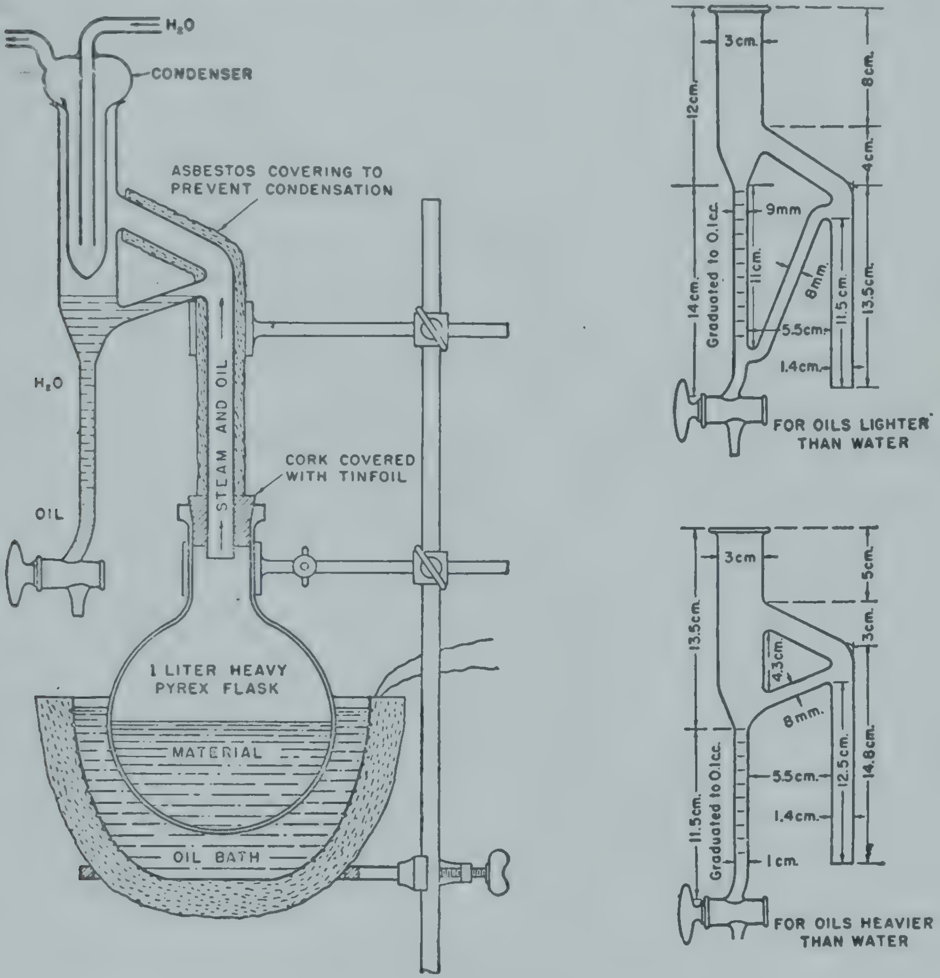

H_2O
CONDENSER
ASBESTOS COVERING TO PREVENT CONDENSATION
H_2O
STEAM AND OIL
CORK COVERED WITH TINFOIL
OIL
1 LITER HEAVY PYREX FLASK
MATERIAL
OIL BATH
3cm.
8cm.
12cm.
4cm.
9mm
14cm.
Graduated to 0.1cc.
11cm.
8mm.
11.5cm.
13.5cm.
5.5cm.
1.4cm.
FOR OILS LIGHTER THAN WATER
3cm
5cm.
13.5cm.
3cm.
4.3cm.
8mm.
14.8cm.
12.5cm.
5.5cm.
1.4cm.
11.5cm.
Graduated to 0.1cc.
1cm.
FOR OILS HEAVIER THAN WATER

FIG. 23. Volatile oil distillation apparatus.

The official methods prescribed for these determinations consist essentially of the separation of the oil from the other components of the drug or oleoresin by means of steam distillation and the accurate measurement of the volume of oil obtained. These operations are carried out in a distillation apparatus designed especially for the purpose (see Fig. 23). All parts that come in contact with the material to be assayed, the volatile oil or steam, are made of glass. The condenser and receiver are combined and are designed to minimize the loss of volatile oil through the escape of vapors and to facilitate the accurate measurement of the volume of oil in the distillate. Receivers that differ slightly from one another in design are employed, depending on whether the oil is lighter or heavier than water (see Fig. 23). Distillation for 4 to 8 hr. is required to ensure complete isolation of the oil.

The chief precaution to be observed in carrying out the determination is not to boil the contents of the flask too rapidly during distillation, as this causes vapors to escape around the condenser and results in a loss of volatile oil.

If the oil in the sample to be assayed is lighter than water, it is measured directly in the receiver, the lower part of which is shaped like a pipette and graduated in tenths of a cubic centimeter. If it is heavier than water, the oil is transferred to a graduated cylinder after distillation is complete, and the water with any remaining oil is drawn off into a small separator. The oil trap is washed with 10 cc. of ether, the ether washings are added to the separator, the mixture is shaken and allowed to separate. The aqueous layer is drawn off and discarded. The ethereal layer is evaporated until the odor of ether is no longer perceptible, the residual oil is drained into the graduated cylinder, and the volume is measured at 25°.

If the oil content is to be determined by weight, a small quantity of anhydrous sodium sulfate is added, the mixture is agitated gently, the clear oil is decanted, and its specific gravity is determined at 25°.

Exercise 84

Object. To Determine the Volatile Oil Content of Clove.

Materials Required. 20 Gm. of ground clove.
About 100 cc. of water.
Volatile oil determination apparatus for oils heavier than water.

Procedure. 1. Place 20 Gm. of the prepared drug in the round-bottomed flask, add 400 cc. of water and mix uniformly. Attach the condenser and separator and boil the contents of the flask slowly during 3 hr., or until distillation is complete.

The drug should be ground to a coarse powder immediately before weighing, and the grinding should be completed as rapidly as possible to avoid excessive loss of volatile oil through evaporation. To ensure complete distillation of the oil, the contents of the flask should be boiled for at least 3 hr.

2. After the distillation is complete, remove the separator, rotate it if necessary to cause oil droplets to settle, and read the volume of oil to 0.1 cc.

The separated oil (sp. gr. 1.038 to 1.060 at 25°) is heavier than water. The volume of oil represents the amount obtained from 20 Gm. of drug. This figure multiplied by 5 will give the yield in cubic centimeters from 100 Gm. of drug.

TABLE 50. OFFICIAL SUBSTANCES WITH VOLATILE OIL CONTENT LIMITS

Substance	Amount used, Gm.	Official requirement per cent, *W/V*	Substance	Amount used, Gm.	Official requirement per cent, *W/V*
U.S.P.			N.F.		
Cinnamon	100	2.5	Cubeb	20	13.0
Cinnamon, Ceylon	100	0.5	Cubeb oleoresin	5	50.0
N.F.			Ginger oleoresin	10	18.0 to 35.0
Anise	100	1.75	Juniper	200	0.5
Buchu	100	1.25	Sassafras	50	4.0
Clove	20	16.0	Thyme	100	1.5
Coriander	200	0.25			

Questions and Problems

1. Consult the official compendiums and explain precautions or procedures to be followed in the volatile oil determination for products containing (*a*) little starch or mucilage; (*b*) considerable starch or mucilage.

2. What official drugs other than clove, assayed for volatile oil content, contain volatile oils that are heavier than water?

3. Why is the correction factor for error due to solubility of the oil-kerosene layer in the aqueous layer 10.5 in compound spirit of orange and only 2.2 in spirit of lavender?

4. Explain each step of the official procedure for the assay of black mustard.

Assay for Volatile Oil in Spirits. The estimation of the volatile oil content of certain official spirits is based upon the separation of the volatile oil by means of an immiscible solvent and measurement of the volume of the oil as illustrated in the following exercise.

Exercise 85

Object. Assay of Peppermint Spirit.

Materials Required. 5 cc. of peppermint spirit.

A Babcock bottle and centrifuge.

A 1-cc. pipette.

1 cc. of kerosene.

50 cc. of a saturated solution of calcium chloride (about 30 Gm. in sufficient water to make 50 cc.) acidified with HCl.

Procedure. 1. "Transfer exactly 5 cc. of Peppermint Spirit to a Babcock bottle, graduated to 8 per cent, add exactly 1 cc. of kerosene from a pipette calibrated to deliver that amount, and mix well. Then add sufficient saturated calcium chloride solution, acidified with hydrochloric acid, almost to fill the bulb of the bottle. Rotate the bottle vigorously to insure thorough mixing, then add sufficient of the calcium chloride solution to bring the separated oil into the neck of the bottle."

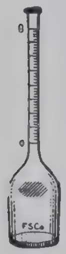


FIG. 24. Babcock bottle.

The acidified calcium chloride solution, when added to the alcoholic spirit, throws the volatile oil out of solution and the oil dissolves in the kerosene when the mixture is shaken. The calcium chloride serves to "salt out" any volatile oil from the diluted alcoholic mixture. The acid ensures the absence of alkali which would render acids, traces of aldehydes, etc., water-soluble. The determination is carried out in a Babcock bottle (Fig. 24), the neck of which is calibrated into eight main divisions of 0.2 cc. each.

2. "Centrifuge for five minutes at about 1500 revolutions per minute, and then read the volume of oil in the stem. Subtract 5 divisions for the kerosene added, and multiply the remaining number of divisions by 4.2 to obtain the volume of peppermint oil in 100 cc. of the Spirit."

The oil-kerosene layer, being lighter than the aqueous layer, rises into the neck of the flask when the mixture is centrifuged. The length of the oil-kerosene layer supplies the information needed for the calculation of the per cent of oil in the spirit; *e.g.*, if 7.4 divisions represents the length of the column of oil-kerosene in the graduated neck of the flask, the oil measures 7.4 − 5.0 = 2.4 divisions because each 5 divisions are equal to 1 cc. (5 × 0.2 cc.) and 1 cc. of kerosene was added. The 2.4 divisions, each of which represent 0.2 cc., are equal to 0.48 cc. of oil. Since a 5-cc. sample is employed, 100 cc. of the spirit yielded 0.48 × 20 = 9.60 cc. of oil. The procedure is subject to slight error, which has been experimentally determined. Consequently, the volume of oil in scale divisions is multiplied by 4.2 (20 × 0.21 cc.) to correct for the error due to solubility of the oil-kerosene layer in the aqueous layer and also due to contraction of volume of the immiscible solvents when mixed. Thus in the example given, 2.4 × 4.2 = 10.08 cc. of oil in 100 cc. of the spirit.

Similar assay procedures are applied to a number of the official spirits in the U.S. Pharmacopoeia and the National Formulary as indicated in Table 51.

TABLE 51. OFFICIAL SPIRITS ASSAYED FOR VOLATILE OIL CONTENT

Spirit	Amount used, cc.	Factor	Official requirement, per cent, *V/V*
U.S.P.			
Orange, compound	2	10.5	25 to 30
Peppermint	5	4.2	9 to 11
N.F.			
Anise	5	4.2	9 to 11
Cinnamon	5	4.2	9 to 11
Lavender	10	2.2	4 to 6
Spearmint	5	4.2	9 to 11

Chapter 15

ALKALOIDAL ASSAYING

Proximate Assays. Alkaloidal assays are commonly referred to as *proximate assays.* When such substances as the alkaloids and glycosides were first isolated from vegetable matter, they were regarded as plant principles that entered immediately into the composition of the plant and that retained their vegetable character unaltered after isolation from the plant. Consequently, they were called *proximate* principles, as opposed to the *ultimate* principles, such as water, carbon, acetic acid, and methyl alcohol, which had previously been obtained more or less from all plant products by the methods of pyroanalysis (destructive distillation).

General Principles. The alkaloidal drugs and preparations derived from them constitute a relatively important group of the official substances that are employed frequently in modern therapy. As a class of medicinal agents, they are characterized by their high potency. A slight deficiency of alkaloid in a preparation may cause a marked decrease in physiological effect; on the other hand, a slight excess may cause toxic effects when the preparation is administered. It therefore follows that the accurate estimation of the quantity of alkaloids present in a medicinal substance is an important subdivision of pharmaceutical analysis.

The assay of alkaloidal drugs and preparations is generally performed for purposes of standardization, proof of purity, commercial evaluation, or pharmaco-legal purposes. Methods of various types have been developed for the quantitative estimation of these principles, *e.g.*, gravimetric, volumetric, colorimetric, potentiometric, and physiological.

The amounts of alkaloids that occur in crude drugs are subject to considerable variation in different samples of the same drug. The variations may be caused by several factors: (1) the age of the plant when it is collected; (2) the season of the year when the drug is harvested; (3) the soil and climate in which the drug is grown; (4) the conditions under which the drug is collected, dried, and stored. The quantity of alkaloid present in galenical preparations is also subject to variation due to a number of factors, some of which are: (*a*) the quality of drug employed; (*b*) the menstruum used in the extraction of the alkaloid; (*c*) the amount of decomposition of the alkaloid during the process of extraction and during the period of storage.

Many alkaloidal preparations deteriorate comparatively rapidly, the rate of deterioration being markedly affected by the nature of the alkaloid, the pH value of the preparation, heat, and light. Frequent restandardization of the alkaloidal drugs and their preparations is therefore essential.

In view of the fact that the alkaloids may comprise only a fraction of 1 per cent of the substance assayed and that this small amount must be separated from numerous other constituents present in the crude drug or preparation, such as resins, volatile and fatty oils, coloring matter, glycosides, fatty acids, gums, and proteins, it is evident that the exact technique involved in any given method must be carefully adhered to in order to estimate the variations in alkaloidal content. It is this special technique that characterizes the chemical assay of alkaloidal drugs, rather than the gravimetric or volumetric nature of the procedure employed.

The principles employed in the quantitative determination of the alkaloids by chemical methods are based upon certain characteristic properties of these substances. The following general properties are possessed by most of the members of this class of compounds: (1) Alkaloids are usually sparingly soluble in water but readily soluble in certain organic solvents that are immiscible with water, such as chloroform, ether, amyl alcohol, benzene, petroleum benzin, or mixtures of these solvents. (2) Alkaloids combine directly with acids to form salts that are usually soluble in water but insoluble in certain organic solvents, such as chloroform and ether. (3) Alkaloids are liberated and usually precipitated from aqueous solutions of their salts by alkalies. (4) Alkaloids form highly insoluble precipitates with a considerable number of reagents, especially with the salts of some of the heavy metals such as mercury, gold, and platinum.

In general, the methods employed to separate the alkaloids from the other constituents present in a drug involve the use of the so-called "immiscible solvents," such as water with ether, water with chloroform, or water with ether-chloroform mixture. The process is generally carried out as follows, the separation of the immiscible solvents being accomplished by means of a separatory funnel (Fig. 25): An accurately weighed portion of the drug is treated with a suitable alkali, whereupon the alkaloids, which usually occur in the drug in combination with acids as salts, are set free. An organic solvent, such as ether, is then added, which dissolves the free alkaloids. An aliquot portion or all of the ethereal solution corresponding to a definite weight of the sample taken is separated and shaken successively with several portions of acidulated water. The complete extraction of the alkaloids from the ethereal solution is determined by testing a portion of the latter with a suitable alkaloidal reagent. The alkaloidal salts formed with the acid dissolve in the water, leaving most of the resins, coloring matter, fatty substances, etc., in the ethereal portion. The acid extractions obtained are combined, made alkaline, and shaken with successive portions of ether. The alkaloids liberated from their salts by the alkali pass into solution in the ether, leaving water-soluble impurities behind. The separated ethereal extractives may then be combined, evaporated to dryness, and weighed; or the residual alkaloid may be dissolved in an excess of acid of known normality, and the excess of acid may be determined by titration with a standard solution of alkali, using a suitable indicator to show when the end point is reached.

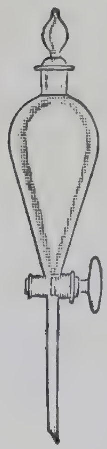


FIG. 25. Separatory funnel.

When the above general procedures are applied in assaying, numerous difficulties may be encountered that will lead to serious error, unless proper precautions are observed, *e.g.*:

1. The organic solvents employed are sometimes appreciably soluble in water; thus, ether is soluble in twelve times its volume of

water at 25°. Consequently, when an organic solvent partially soluble in water, such as ether, is employed to extract the alkaloid from an aqueous solution, some of the alkaloid will remain in the aqueous solution. This difficulty may be overcome by extraction with small successive portions of the immiscible solvent.

2. Resins, fats, and many other organic substances remain in solution in the organic solvent if the aqueous liquid employed in the shaking-out process is acid, but they dissolve in the aqueous liquid if it is alkaline, forming soaps, resinates, etc. Care should be exercised, therefore, to have sufficient acid in the aqueous solution used to react with the alkaloid from the organic solvent and also to neutralize all the alkali and leave a slight excess of acid.

3. When the liquids are shaken in the separatory funnel, emulsification may occur between the organic solvent and the aqueous solution, making it exceedingly difficult or impossible to separate the liquids quantitatively. The formation of emulsions may frequently be avoided by rotating the separatory funnel containing the liquids slowly and occasionally inverting it in place of shaking vigorously. Since the rapidity with which the alkaloids are extracted from a liquid in which they are insoluble, by an immiscible liquid in which they are soluble, is largely dependent on the rate of change of the interface between the liquids, it is necessary that gentle agitation be continued longer than is required when strong agitation is employed. If emulsification occurs, it is often possible to break the emulsion by some one or more of the following procedures: (*a*) addition of more of the aqueous or organic solvent; (*b*) gentle stirring with a glass rod; (*c*) drawing off the separated solvent as rapidly as it separates; (*d*) addition of a few drops of alcohol; (*e*) addition of a few cubic centimeters of salt solution; (*f*) addition of acid, if alkaline; (*g*) addition of alkali, if acid; (*h*) filtration through a pledget of cotton; (*i*) drawing off the emulsion and treating it with anhydrous sodium sulfate. If none of these methods suffices to break the emulsion, it may be necessary to start the assay over. Time is always saved by taking every precaution to prevent the formation of emulsions. Emulsions formed in the assay of drugs containing a large proportion of fatty matter should be treated according to the following Pharmacopoeial directions:

"In such cases it is advisable to add sufficient sulfuric acid to assure acidity, and to evaporate the volatile solvent, while stirring with a glass rod. When the resinous and fatty matter has been agglutinated, cool the acid solution and filter it through a small, wetted filter into a separator. Redissolve the residue in 15 cc. of ether, add 5 to 10 cc. of $0.1N$ acid, evaporate the ether as before, with continued stirring, and pour the acid solution through the filter into the separator. Repeat the extraction of the fatty residue with dilute acid two or three times and finally wash the filter free from alkaloids."

4. When an acid solution, contained in a separatory funnel to which ether has been added, is made alkaline, sufficient heat is frequently generated to volatilize some of the ether and cause considerable pressure in the funnel. Often the pressure generated is sufficient to force the stopper out of the funnel with loss of some of its contents, particularly when the funnel is shaken. For this reason, it is advisable to permit the mixture to stand and cool for a short time after adding the alkali and to agitate it gently at first.

Sources of Error. Numerous possibilities of error are encountered in alkaloidal assaying other than those common to all analytical work, such as those caused by inaccurate weighing, faulty calibration and reading of measuring apparatus, incorrect strength of volumetric solutions, dirty apparatus, etc. Some of the more common errors are caused by: (1) failure to secure complete extraction of the alkaloids from the drug or from solution; (2) loss of volatile solvent during maceration of the drug before the aliquot portion is taken; (3) imperfect separation of the immiscible liquids; (4) failure to wash the stems of separators and funnels, graduates, and other apparatus with solvent to recapture small amounts of alkaloidal residues; (5) decomposition of the alkaloids, especially those which have an ester structure, upon prolonged contact with strong acids and alkalies; (6) use of the wrong indicator.

Other sources of error, such as variation in the moisture content of the sample, frequently cause different analysts to obtain nonconcordant results in the assay of the same drug. The most common cause of variations in results obtained by different workers, however, is referable directly to a lack of familiarity with the procedures involved, since considerable experience in making assays of this type is essential to acquire proficiency.

Theory of Distribution Coefficient. According to Nernst's law, if two practically immiscible solvents are in contact and a substance that is soluble in both liquids is added to them, the substance will distribute itself between the liquids in such a way that the ratio of the concentrations of the two solutions is a constant irrespective of the quantity of solute. This constant is variously termed the *distribution ratio, distribution constant, distribution coefficient*, or *partition coefficient.*

If C_1 is used to designate the concentration of an alkaloid in ether, and C_2 to designate the concentration of the same alkaloid in an equal volume of an aqueous acid solution, Nernst's law may be formulated $C_2/C_1 = K$, where K is the distribution constant or coefficient. If there are several solutes, such as alkaloids, resins, etc., present in the ether when it is shaken with the acid solution, the distribution of each solute is the same as if it were present alone.

Measurable deviations from Nernst's law take place in alkaloidal assaying owing to molecular association and dissociation, ionization, chemical reaction, etc., but the fundamental principle that it expresses may be used to explain why it is better to extract the alkaloid from a solvent with several small portions instead of one large portion of an immiscible solvent; *e.g.*, assume that 1 Gm. of atropine in 100 cc. of ether solution when shaken with an equal volume of dilute sulfuric acid distributes itself so that 0.1 Gm. remains in the ether layer and 0.9 Gm. in the acid layer of the solvents, $C_{\text{acid}}/C_{\text{ether}} = 9$ (the distribution coefficient). Obviously, only 90 per cent of the atropine present would be extracted from the ethereal solution by extracting it once with 100 cc. of dilute sulfuric acid solution. If the extraction is performed with 200 cc. of the acid solution, the amount of atropine extracted should be $\dfrac{x/200}{\dfrac{1-x}{100}} = 9$, and $x =$ 0.947 Gm. of atropine.

Where x = the amount of atropine extracted from the ether by the acid solution, $C_{\text{acid}} = \dfrac{x}{200}$, and $C_{\text{ether}} = \dfrac{1-x}{100}$. If the original solution is extracted with several small portions of acid solution, *i.e.*, 50 cc., the amount of alkaloid extracted will be $C_{\text{acid}} = \frac{x}{50}$ and $C_{\text{ether}} = \frac{1 - x}{100}$ and since $\dfrac{x/50}{\frac{1 - x}{100}} = 9$, $x = 0.818$ Gm. of atropine removed by the first extraction with acid, leaving 0.182 Gm. in the ethereal layer. A second extraction with a 50-cc. portion of acid solution will remove $\dfrac{x_1/50}{\frac{0.182 - x_1}{100}} = 9$ and $x_1 = 0.1489$ Gm. of atropine where x_1 equals the amount of atropine that passes into the acid solution. Thus, in two extractions, $0.818 + 0.1489 = 0.9669$ Gm. of atropine are removed from the ethereal solution by 100 cc. of acid solution, whereas a single extraction with 200 cc. of acid solution removed only 0.947 Gm. of the alkaloid.

Since economy in the use of solvents is an important consideration and large volumes are difficult to handle in shaking out the alkaloids, it is more practical to use several small portions of immiscible solvent than one large portion. In the official assay processes, 10- to 20-cc. portions of the immiscible solvent are used because it has been found that these quantities serve best to extract the alkaloids quickly and economically.

Choice of Indicators for Alkaloidal Titrations. In the official alkaloidal assays by volumetric methods, the use of methyl red solution is recommended in all the titrations. The same lot of indicator used in the standardization of the volumetric solutions should be employed in titrating the alkaloids. Numerous investigators have found that an indicator other than that employed in the official assays gives more accurate results. Table 52, showing the indicators best suited to the titration of some of the more important alkaloids, is taken with slight modification from a paper by H. Wales.[1]

Materials Required. In the statement of the materials required under the following respective exercises, it is assumed that the

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[1] Wales, H., *J. Ind. Eng. Chem.*, **18**, 390, 1926.

TABLE 52. INDICATORS FOR ALKALOIDAL TITRATIONS

Salt of	Average pH	pH range for indicator	Indicator
Aconitine	5.04	4.2 to 5.8	Methyl red
Arecoline	4.81	3.8 to 5.8	Methyl red
Atropine	5.56	3.8 to 7.2	Methyl red
Brucine	4.85	3.9 to 6.0	Methyl red
Cephaeline	4.81	4.2 to 5.4	Methyl red
Cinchonidine	5.90	5.4 to 6.4	Bromocresol purple
Cinchonine	6.02	5.5 to 6.5	Bromocresol purple
Cocaine	5.20	4.0 to 6.5	Methyl red
Codeine	4.86	3.6 to 6.3	Methyl red
Cotarnine	5.97	4.9 to 7.0	Bromocresol purple
Diacetyl morphine	4.89	4.2 to 5.7	Methyl red
Emetine	4.90	4.2 to 5.6	Methyl red
Ethyl hydrocupreine	6.33	5.6 to 7.0	Bromocresol purple
Ethyl morphine	4.99	4.2 to 5.8	Methyl red
Homatropine	5.74	3.9 to 7.6	Methyl red, propyl red, bromocresol purple
Hydrastine	4.45	3.8 to 5.0	No end point with methyl red or bromophenol blue
Hyoscine	4.83	3.6 to 5.6	Bromophenol blue
Hyoscyamine	5.83	3.8 to 7.6	Methyl red, propyl red, bromocresol purple
Morphine	4.68	4.0 to 5.2	Methyl red
Narcotine	4.43	3.9 to 4.9	Bromophenol blue
Nicotine	5.26	4.4 to 6.1	Methyl red
Papaverine	4.23	3.8 to 4.6	Indistinct, cannot be titrated
Physostigmine	4.85	3.8 to 6.0	Methyl red
Pilocarpine	4.31	3.6 to 5.0	Bromophenol blue
Quinidine	6.10	5.5 to 6.5	Bromocresol purple
Quinine	6.12	5.5 to 6.5	Bromocresol purple
Strychnine	4.81	3.8 to 6.0	Methyl red
Thebaine	5.08	4.0 to 6.2	Methyl red
Yohimbine	4.72	4.0 to 5.3	Methyl red

student's locker is supplied with the four separatory funnels necessary to conduct alkaloidal assays in duplicate, as well as measuring cylinders, burettes, flasks, etc. It is also assumed that the following substances are available in the laboratory: reagent sulfuric acid, hydrochloric acid, Valser's reagent, ammonia water, distilled water, alcohol, mercuric iodide test solution, iodine test solution, and methyl red T.S. The quantities of immiscible organic solvent required are approximate.

Alkaloidal Test Solutions. A number of reagents form insoluble compounds with many of the alkaloids, precipitating the latter from aqueous solution. In many cases, the presence of a mere trace of alkaloid can be detected by adding a suitable reagent to its solution. Some of the frequently used reagents are auric chloride, platinic chloride, mercuric chloride, gallotannic acid, picric acid, phosphomolybdic acid, potassium mercuric iodide, mercuric iodide, and iodine in potassium iodide. Although most of these reagents are employed in qualitative tests for the alkaloids, only the last two are of importance in the official assays, since they are employed to test for the complete extraction of the alkaloids by immiscible solvents.

Mercuric Iodide Test Solution, commonly known as *Valser's reagent*, is prepared by slowly adding a 10 per cent solution of potassium iodide to red mercuric iodide until almost all of the red mercuric iodide is dissolved. The excess mercuric iodide is then removed by filtration. This reagent forms white precipitates with minute traces of many of the alkaloids, when it is added to their acidified aqueous solutions. The composition of the precipitates usually varies somewhat depending upon the conditions under which the precipitation is conducted.

Iodine test solution, commonly known as *Wagner's reagent*, is a solution containing approximately 1.275 Gm. of iodine and 3.6 Gm. of potassium iodide dissolved in sufficient distilled water to make 100 cc.; 0.1N iodine solution may be employed. Wagner's reagent yields reddish or red-brown precipitates of complex composition with nearly all the alkaloids, even in dilute solutions, when it is added to an aqueous solution slightly acidified with sulfuric acid. The precipitates are usually amorphous. The quantity of the reagent used should not be sufficient to color the solution more than a faint yellow.

General Procedures. 1. *Selection of the Sample.* The sample of a crude drug taken for assay must represent the entire drug and be powdered to a specified fineness. Usually the drug must be ground to a fine powder (No. 60), but finer powders may be employed. When the whole crude drug, such as leaves or seeds, is powdered for assay, care must be exercised not to exclude leaf stems, seed coats, etc., since the alkaloid may be localized in amounts varying in the different morphological parts of the drug. Care should be taken to avoid the loss of water during the powdering of the drug. If it is impossible to avoid this loss, the drug should be dried at a low temperature before powdering, the loss of water noted, and a correction made in the final calculations.

2. *Weighing of Portions for Assay.* In the preparation of drugs for assay, all portions directed to be weighed should be weighed accurately, but with crude drugs, accuracy to within 10 mg. for quantities of 5 Gm. and over is sufficient. Portions of pilular extracts may be weighed on a piece of waxed or parchmentized paper, the surplus paper cut away, and the extract and adhering paper dropped into a separator, beaker, or dish containing the solvent, and the extract dissolved. In transferring weighed portions to a separator, the dish containing the material to be assayed should be thoroughly rinsed and the rinsings should be added to the separator.

3. *Methods of Extraction.* The alkaloids are extracted from drugs by the process of maceration, by the process of percolation, and by the process of continuous extraction. The maceration process is adapted to those drugs which contain a relatively large amount of alkaloids and which, because of their nature or the nature of the drug, are difficult to extract completely by the process of percolation. In the maceration process, it is assumed that all the alkaloids are dissolved in the organic solvent and that an aliquot part of the solvent contains an amount of the extracted alkaloids in definite proportion to the weight of drug taken as sample. Many analysts believe that the aliquot portion of solvent obtained by the maceration process does not contain exactly the assumed proportion of the total alkaloids. Consequently, methods for the total extraction of the alkaloids are regarded as more satisfactory.

a. By Maceration. An accurately weighed portion of the ground drug is treated with the specified solvent or mixture of solvents, made alkaline with ammonia T.S., and thoroughly mixed. It is then allowed to macerate for 12 to 24 hr. with occasional agitation, or for a shorter period with continuous agitation. At the end of this period, the drug is allowed to settle, an aliquot portion of the solvent decanted, and treated as described for the purification of the alkaloids.

The organic solvent, accurately measured from a calibrated cylindrical graduate, is added and allowed to stand in contact with the powdered drug so that the small particles of the drug will be impregnated with the organic solvent before the ammonia is added. If the ammonia test solution is added first, the drug particles become wetted by the water with which the organic solvent is immiscible, and the latter will not permeate the drug cells to extract the alkaloids completely. The alkaloids generally occur in combination with various acids as salts in the crude drug. The ammonia reacts with these alkaloidal salts, forming the free alkaloids and the ammonium salts of the acids, and the liberated alkaloids dissolve in the organic solvent. The process of extraction of the alkaloid from the cellular structure of the drug proceeds slowly, probably because it is an osmotic process. Consequently, a period of maceration is required to permit the complete transference of the alkaloids from the drug cells to the organic solvent. The rate of extraction is accelerated by grinding the drug to a fine powder, thereby breaking down its cellular structure, and by frequent agitation of the drug-solvent mixture, so that the solvent in contact with the particles is changed frequently. The small particles of drug, which sometimes remain in suspension in the organic solvent, become hydrated when the latter is shaken with a little water and settle out. The flask in which the maceration and agitation of the drug with the solvents are carried out should be tightly stoppered throughout the entire period of maceration to prevent the escape of volatile organic solvent, since the volatilization of some of the solvent might cause a considerable error; *e.g.*, if 100 cc. of solvent is taken for the extraction of the alkaloids from a drug and 10 cc. is lost by volatilization, and an aliquot portion of 50 cc., representing one-half of the weight of the sample is measured for assay, the aliquot portion will contain five-ninths instead of one-half of the alkaloids contained in the drug, and an error of approximately 5 per cent will be caused in the assay result.

An aliquot portion of the supernatant organic solvent is poured off from the mixture and measured accurately in a calibrated cylinder; *e.g.*, if 4 Gm. of a drug is macerated with 100 cc. of solvent and a 50-cc. portion of the solvent is decanted, the 50-cc. portion of solvent will contain an amount of extractive equivalent to $^{50}/_{100} \times 4 = 2$ Gm. of the drug, provided that none of the solvent originally added is lost by volatilization. The aliquot portion of solvent should be measured at the same temperature at which the original solvent was measured, since the volume of liquids varies somewhat with changes in temperature.

b. By Percolation. An accurately weighed quantity of the ground drug is placed in a suitable container and completely saturated with the specified solvent or mixture of solvents, and allowed to stand for 5 min. A quantity of ammonia T.S. sufficient to make the mixture distinctly alkaline is added and thoroughly mixed with the drug. The mixture is then transferred to a cylindrical percolator, previously prepared by packing the outlet with purified cotton. A small amount of the solvent may be used to rinse the container and the rinsing added to the percolator. The drug is allowed to macerate for a suitable period of time (from 1 to 12 hr. or overnight, depending upon the drug to be assayed). Then the drug is firmly packed, a pledget of purified cotton placed above it, and percolated slowly with the solvent until it is completely exhausted of its alkaloid contents. Complete extraction of the alkaloid is determined by evaporating about 4 cc. of the last percolate to dryness, dissolving the residue in 0.5 cc. of approximately 0.5*N* acid and adding a drop of mercuric iodide T.S. (Valser's reagent), or, when testing for caffeine or colchicine, a drop of iodine T.S.; not more than a slight turbidity should be produced. The percolate is then treated for the purification of the alkaloids.

The extraction of the alkaloids from a drug by cold percolation has the disadvantage that it requires a relatively large amount of solvent to effect complete extraction. The cold percolation process of extraction is best suited to the extraction of drugs that contain unstable alkaloids or that yield a large amount of nonalkaloidal extractive by hot percolation. The cold percolation process is well suited to the extraction of those drugs which contain a comparatively small percentage of alkaloids and which can be readily extracted by percolation. The importance of the total extraction of the alkaloid from a drug such as hyoscyamus becomes apparent when it is recalled that the official drug is required to contain only 0.040 per cent of alkaloids.

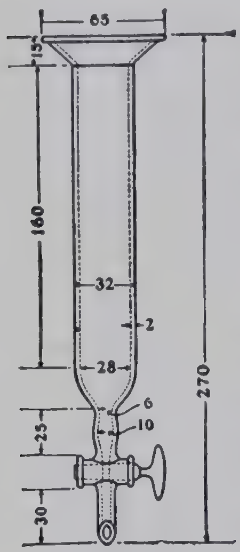

Fig. 26. Percolator recommended for type process B (measurements are given in millimeters).

The percolator employed in the extraction of the drug should be of a special type similar to that illustrated in Fig. 26. The barrel of the percolator should be about 15 or 16 cm. long and about 3 or 4 cm. in diameter. A percolator of these dimensions will accommodate from 10 to 25 Gm. of drug, and, because of the small diameter, a long column of the drug will be provided through which the menstruum must pass. The glass stopcock is necessary to eliminate the use of rubber connections, since rubber is dissolved to some extent by chloroform and ether.

The alkaloids are liberated by the ammonia, and they dissolve in the organic solvent as described under (*a*).

c. By Continuous Extraction. An accurately weighed portion of the ground drug is placed in an extraction thimble and the thimble inserted into a suitable extractor (a Soxhlet extractor of appropriate size is satisfactory). The drug is moistened with the specified solvent and mixed by means of a stirring rod and allowed to stand about 5 min. It is then made alkaline with the specified quantity of ammonia T.S. and thoroughly mixed. The stirring rod is rinsed with a small portion of the solvent and the drug macerated for 6 to 12 hr. or overnight. The drug is then packed in the thimble, covered with a pledget of purified cotton, a sufficient quantity of solvent is added, and the drug extracted for a specified period of time or until completely extracted. The solvent remaining in the extracion chamber is transferred to the receiving flask and the liquid xtract treated for the extraction of alkaloids.

An extraction apparatus of the type illustrated in Fig. 19 and described on page 263 is very satisfactory. The continuous extraction process requires a small amount of the organic solvent and, in most cases, complete extraction of the alkaloids is obtained in from 3 to 5 hr.

4. *Extraction of the Alkaloids from Organic Solvents with Acid.* The alkaloids are extracted from ethereal or chloroformic solutions by shaking with dilute acids. The concentration and quantity of the acid employed are left to the discretion of the analyst, but the use of 10 cc. of a 1*N* or 5 per cent acid or sufficient to render the mixture distinctly acid for the first extraction and of 10-cc. portions of 0.5*N* or 2 per cent acid for succeeding extractions will be found to be practical and convenient. When the drug contains a large amount of fat, the use of a smaller volume of more concentrated acid for the first extraction may be advantageous in preventing emulsions. The shaking of the immiscible solvents should not be violent, but it should be sufficient to mix them thoroughly without emulsification. Repeated successive extractions with small portions of the acid must be continued until the alkaloid is completely removed from the ethereal or chloroformic liquid, as shown by tests with the designated reagents. Each portion of the acid solution should be carefully separated from the organic solvent and run directly into another separatory funnel. In all assays, the extraction should be continued until 0.5 cc. of the last acid washing shows no more than a slight turbidity on the addition of a drop of mercuric iodide T.S., or, in the case of caffeine and colchicine, on the addition of a drop of iodine T.S. If traces of organic solvent are drawn off with the acid solution, they may be removed by washing the combined acid extractives with 2 to 3 cc. of ether or chloroform, which is separated and washed in turn with about 5 cc. of distilled water, the aqueous washings being separated and added to the acid solution.

If sulfuric acid is used, the acid converts the free alkaloids dissolved in the organic solvent into their sulfates, which pass into solution in the aqueous acid layer of the immiscible solvents when the mixture is shaken, leaving chlorophyll, resins, fixed and volatile oils, and other impurities dissolved in the organic solvent. The rate at which the alkaloids pass from solution in the organic solvent into the acid solution is increased greatly by shaking the mixture, since a greater area of contact surface between the liquids is produced. Sulfuric acid solution is employed to extract the alkaloids, because the ammonium sulfate formed in the next step in the procedure is not appreciably soluble in the organic solvent used for the final extraction of the alkaloid.

5. *Extraction of the Alkaloids from Acid Solution with Organic Solvents.* The acid solution of the alkaloids contained in a separatory funnel is made alkaline, in most cases, with ammonia T.S., and then shaken with several successive portions of organic solvent, such as ether, chloroform, or ether-chloroform mixture. The volume of organic solvent used in each extraction should be at least one-half that of the aqueous solution. The extraction with portions of organic solvent must be repeated as long as any alkaloid is extracted by the solvent. To determine the completion of extraction, evaporate 1 cc. of the last washing and dissolve the residue in 0.5 cc. of approximately $0.5N$ hydrochloric acid; the resulting solution should show no more than a slight turbidity on the addition of a drop of mercuric iodide T.S., or, in the case of caffeine and colchicine, on the addition of a drop of iodine T.S. The number of extractions required depends largely on the character of the alkaloid. With most alkaloids, it is advisable to extract three or four times before testing. Physostigmine and pilocarpine require about twice as many extractions as other alkaloids.

The ammonia reacts with the alkaloidal sulfates contained in the aqueous solution to form the free alkaloids and ammonium sulfate. If considerable quantities of alkaloid are contained in the acid solution, the alkaloids may be precipitated when the alkali is added. Consequently, the first portion of organic solvent is added prior to the addition of the ammonia water to dissolve any alkaloidal precipitate formed. If this procedure is not followed, the precipitate frequently agglomerates into large particles which dissolve slowly when the organic solvent is added.

6. *Evaporation of Organic Solvents.* The evaporation of the final alkaloidal solution in organic solvents may be performed by distilla-

ion with recovery of the solvent, by evaporation on a steam bath or
and bath, or in a current of warm air. When the evaporation is
onducted on a steam bath, precautions must be observed to prevent
he ignition of the vaporized solvent. The operation may be per-
ormed by heating a prepared water bath until the water boils,
urning out the flame, and placing the alkaloidal solution, contained
a suitable container, on the bath. If a sand bath is employed,
should be preferably of the steam-heated type, and the tempera-
ure of the bath should be about 60°.

Chloroform tends to form unstable compounds with many alka-
oids: consequently, when chloroformic solutions of the alkaloids
re evaporated, the residue may retain traces of chloroform rather
enaciously. These traces of chloroform are best removed by treat-
ng the alkaloidal residue with 2 to 3 cc. of ether or alcohol and evap-
rating the solution to dryness again. This is a wise precaution to
mploy in the evaporation of all solutions of alkaloids in organic
olvents, when the final determination is made by volumetric
nethods in order to remove small traces of ammonia that may be
ccluded in the alkaloidal residue, particularly if the latter is gummy
n nature.

Those alkaloids which have the structure of esters, such as atro-
pine, hyoscyamine, and cocaine and some others are decomposed
nore or less by heat and by prolonged contact with water and strong
cids and alkalies. Consequently, their solutions should be evapo-
ated at a low temperature, usually at about 60°; and, if it is neces-
sary to allow the alkaloid in solution to stand from one laboratory
period to the next before the assay can be completed, it should be
shaken out of acid or alkali solutions into an organic solvent and
ept in a tightly stoppered flask.

7. *Gravimetric Determinations of Alkaloids.* If the alkaloid is to
e estimated gravimetrically, the solvent is evaporated in the usual
nanner from a previously weighed evaporating dish, wide-mouth
lask, or beaker, and the residue is dried to constant weight in an
lectric or hot-air oven at 100 to 110° and weighed. The same pro-
edure should be followed in drying the flask or beaker used as is
mployed in drying the container with its residue. When the resi-
due is to be weighed, if the final solvent has been chloroform, the

last traces of that solvent should be removed by the addition of a little neutral ether or alcohol, followed by evaporation. Care must be taken to avoid loss by decrepitation, especially when evaporating chloroformic solutions of nux vomica or cinchona alkaloids. Decrepitation may usually be prevented by the addition of a little alcohol after the solution has been reduced to a volume of 1 or 2 cc., evaporating at a low temperature, and rotating the container during the process.

8. *Volumetric Determinations of Alkaloids.* If the alkaloid is directed to be assayed volumetrically, the residue should be softened by the addition of about 1 cc. of neutral alcohol or ether, the required amount of standard acid added, and the mixture gently warmed to insure the complete solution of the alkaloid. If preferred, the alkaloidal residue may be dissolved in chloroform, the standard acid added, and the chloroform removed by evaporation. Before titrating add a sufficient quantity of distilled water to make the volume of the mixture measure about 25 cc. The amount of acid in excess of that which combines with the alkaloid is then determined at once by titration with standard alkali.

As a rule, the volumetric method of determining the quantity of alkaloid obtained in a given assay is more accurate than the gravimetric method, since many impurities that cannot be removed are present in the alkaloidal residue and are computed as alkaloid when the residue is weighed. If the residue is titrated, however, only those impurities which are acidic or basic affect the result.

Absorbents. In assaying fluidextracts, tinctures, and other preparations of alkaloid-bearing drugs, it is often necessary to evaporate these to dryness and, to avoid loss and to aid in the evaporation, they are usually added to some absorbent material. Paper pulp or asbestos fiber should be used for this purpose. Such absorbent material must be acid- and alkali-washed and then rendered neutral by washing with distilled water and dried before being used.

Apparatus for Proximate Assays. When a container of definite size and shape is recommended in a proximate assay process, it is understood that this is advisory and not obligatory, except when volumetric flasks, measuring burettes, or other exact measuring apparatus are specified.

Chapter 16

OFFICIAL TYPE METHODS

The official alkaloidal drugs and the galenical preparations con-aining alkaloids are assayed by two type methods and by special nethods. The type methods are (1) the aliquot part method for rugs and galenical preparations and (2) the total extraction method or drugs and galenical preparations. The procedures in the following assays are taken almost verbatim from the U.S.P. and the N.F., light changes being introduced where necessary to include more pecific details.

Gravimetric Assays by the Aliquot-part Method

Exercise 86

Object. Assay of Hydrastis for Ether-soluble Alkaloids.

Materials Required. 10 Gm. of hydrastis in No. 60 powder.
200 cc. of ether.

Procedure. 1. "Place 10 Gm. of Hydrastis, in fine powder and accurately veighed, into a suitable container, add 100 cc. of ether, allow the mixture to tand about 5 minutes, and add 10 cc. of ammonia T.S. Shake the mixture for hour in a mechanical shaker or during 2 hours intermittently, and then set it side overnight. Again shake it during 30 minutes, allow the drug to settle. nd decant 50 cc. of the clear ether solution." (See General Procedure 3*a*, page 332.)

The alkaloids found in the rhizomes and roots of hydrastis consist hiefly of hydrastine and berberine in varying proportion. The atter is almost inactive, while the hydrastine is very active physiologically. Consequently, a determination of the total alkaloids of ydrastis would not be a correct measure of the therapeutic activity f the drug. Ether is used, therefore, as the organic solvent in the xtraction of the alkaloids, because hydrastine is readily soluble and erberine is practically insoluble in this solvent. The aliquot portion of 50 cc. of the ethereal solution is equivalent to $^{50}/_{100} \times$ weight in grams of the sample of hydrastis.

2. "Extract the alkaloids from the ether solution by shaking with 5 successive 20-cc. portions of dilute sulfuric acid (about 1 per cent). Combine the acid solutions in a separator, add about an equal volume of ether, then add a slight excess of ammonia T.S., and immediately shake to extract the alkaloids." (See General Procedure 4, page 335.)

The sulfuric acid converts the hydrastine into hydrastine sulfate, which is soluble in the acid solution.

3. "Separate the ether layer, and complete the extraction of the alkaloids from the alkaline aqueous layer by shaking with at least 6 successive 20-cc. portions of ether. A 5-cc. portion of the last ether extraction upon evaporation and drying at 105° for 2 hours, must leave a residue of not more than 0.6 mg." (See General Procedure 5, page 336.)

The hydrastine, liberated from its sulfate, is practically insoluble in water, but it dissolves in ether to the extent of 1 Gm. in 175 cc. at 25°. Traces of berberine carried over into the acid solution are eliminated by using ether as the immiscible solvent, since berberine is soluble in water (1 Gm. in 4.5 cc. at 22°) and insoluble in ether.

4. "Filter the ether extracts and evaporate the ether. Dry the residue at 105° for 2 hours."

"The weight obtained represents the yield of anhydrous, ether-soluble alkaloids from 5 Gm. of Hydrastis." (See General Procedures 6 and 7, pages 336 and 337.)

The percentage of ether-soluble alkaloids contained in the root is found by simple calculation as follows:

$$\frac{\text{Wt. of alkaloidal residue}}{0.5 \times \text{wt. of hydrastis}} \times 100 = \text{per cent}$$

The National Formulary requires that hydrastis contain not less than 2.5 per cent of ether-soluble alkaloids. Compare the percentage of alkaloids found in the sample assayed with the official requirement.

The fluidextract and tincture of hydrastis are assayed by procedures similar to that employed in the assay of hydrastis.

Questions and Problems

1. Why is hydrastis assayed for its content of ether-soluble alkaloids?

2. Explain in detail how to test for hydrastine in the ethereal solution that as been shaken out with acid.

3. Can hydrastine be estimated volumetrically (see table of indicators, page 29)?

4. What is an aliquot portion of a solution?

5. Why is it more practical to use a number of small portions of immiscible olvent in shaking out the alkaloids than to use one or two large portions?

Exercise 87

Object. Assay of Cinchona for Total Alkaloids.

Materials Required. 5 Gm. of cinchona in No. 60 powder.

200 cc. of ether-chloroform mixture.

100 cc. of chloroform.

Procedure. 1. "Place 5 Gm. of Cinchona, in fine powder, and 15 cc. of 3 per cent hydrochloric acid in a 500-cc. flask and heat the mixture on a water bath for 1 hour. Cool and add 200 cc. of ether-chloroform solution (ether 3 volumes, chloroform 1 volume) and 10 cc. of stronger ammonia T.S. Stopper he flask tightly and shake it for 1 hour in a mechanical shaker. Allow the nixture to stand over night, again shake it for 30 minutes, and then allow the lrug to settle. (If the supernatant liquid is not clear, add a few cubic centineters of distilled water, again shake the contents of the flask vigorously, and llow the drug to settle.)" (See General Procedure 3*a*, page 331.)

Cinchona bark contains about 20 alkaloids, the most important of which are quinine, quinidine, cinchonine, and cinchonidine. The alkaloids occur as salts in combination with quinic and cinchotannic acids. These salts are decomposed when boiled with hydrochloric acid, with the formation of the corresponding alkaloidal hydrochlorides that are soluble in the water. When the aqueous solution is made alkaline with ammonia, the liberated alkaloids pass into solution in the ether-chloroform mixture almost completely, because of the large volume of the latter employed.

2. "Quickly decant 160 cc. of the clear, ether-chloroform solution, measured at approximately the same temperature as the original ether-chloroform solution and representing 4 Gm. of drug." (See General Procedure 3, page 331.)

The aliquot portion is equivalent to ${}^{160}/_{200}$ × the weight of the sample taken.

3. "Transfer the solution to a separator, rinse the measuring vessel with a small quantity of the original menstruum and add the rinsings to the separator. Completely extract the alkaloids with approximately 5 per cent sulfuric acid, and collect the acid solution of the alkaloids in a second separator." (See General Procedure 4, page 335.)

The alkaloidal sulfates are formed and dissolve in the acid solution.

4. "Make the acid solution strongly alkaline with ammonia T.S., and completely extract the alkaloids with chloroform. Evaporate or distil the chloroform in a tared beaker or flask and dry the alkaloidal residue at 105° for 2 hours. The weight obtained, multiplied by 25, represents the per cent of the alkaloids of Cinchona in the drug." (See General Procedures 5, 6, and 7, pages 336 to 338.)

The ammonia water reacts with the alkaloidal sulfates, setting the alkaloids free and forming ammonium sulfate. Only a slight excess of ammonia should be used to make the solution alkaline, since the cinchona alkaloids are appreciably soluble in strong ammonia solution. The alkaloids set free by the alkali dissolve in the chloroform.

It has been reported that the cinchona alkaloids form addition products with chloroform, which are not entirely broken down when the residue from the chloroformic solution of the alkaloids is dried at 100°. The error in the assay results caused by the retention of chloroform by the alkaloidal residue when the latter is dried as directed, however, is negligible.

The gravimetric method is employed in the determination of the alkaloids in the residue, because the indicator end point is not sharp when the mixture of alkaloids is titrated.

The N.F. requires that cinchona contain not less than 5 per cent of total alkaloids when assayed by the above method. Calculate the percentage of alkaloids found in the sample assayed and compare the results with the official requirement.

Questions and Problems

1. Why is hydrochloric acid used in the initial menstruum to extract the alkaloids from cinchona?

2. Does the ether-chloroform mixture extract practically all the alkaloid present in the sample in the first step?

3. Why should the addition of a large excess of ammonia to the acid solution of the alkaloids previous to the shaking out with chloroform be avoided?

Exercise 88

Object. Assay of Compound Cinchona Tincture

Materials Required. 50 cc. of compound tincture of cinchona.
200 cc. of chloroform.
200 cc. of ether.

Procedure. 1. "Accurately measure 50 cc. of Compound Cinchona Tincture and evaporate it, at a temperature not exceeding 100°, to a volume of about 10 cc. Add sufficient asbestos fiber or paper pulp to adsorb the liquid, and continue the evaporation to dryness. Transfer the residue to a flask or bottle, add 200 cc., accurately measured, at room temperature, of ether-chloroform mixture (ether 4 volumes, chloroform 1 volume) and sufficient ammonia T.S. (which may be used to rinse out the adhering portions of the Tincture from the evaporating dish) to render the mixture strongly alkaline. Securely stopper the container and shake mechanically during 1 hour, or intermittently during 2 hours, and then allow the mixture to stand overnight."

The tincture is evaporated to drive off the alcohol, which is miscible with ether and chloroform. An adsorbent such as asbestos fiber or paper pulp (filter paper torn into small pieces) is added, so that the nonvolatile residual matter, consisting largely of alkaloidal hydrochlorides, glycerin, and cinchona red, will be deposited as a thin film when the tincture is evaporated. If some adsorbent is not used, a resinous, varnish-like residue usually results, from which the alkaloids cannot be extracted readily. The addition of ammonia T.S. liberates the alkaloids that dissolve in the ether-chloroform mixture along with the glycerin, cinchona red, and considerable other extractive matter.

2. "Again shake the mixture intermittently for 30 minutes, allow to settle, quickly decant 160 cc. (representing 40 cc. of the Tincture) of the approximately clear liquid. Filter this into a separator and wash the measuring vessel with sufficient of the ether-chloroform mixture, adding the rinsings to the filter. Extract the alkaloids from the clear liquid with acidified water, using sufficient diluted sulfuric acid to render the contents of the separator and each extract distinctly acid to litmus paper. Pass the acid extracts in succession through a moistened, double filter into a second separator. Render the combined liquids distinctly alkaline with stronger ammonia T.S., and extract with chloroform. Pass the chloroform extracts through a double filter, which is kept saturated with chloroform, into a suitable, tared receptacle. Evaporate the chloroform on a water bath, dry the residue at 105° for 2 hours, and weigh. The weight multiplied by 2.5 indicates the weight of alkaloids in 100 cc. of the Compound

Cinchona Tincture." (See Assay of Cinchona, Procedures 2, 3, and 4, pages 331 to 336, for explanation.)

Volumetric Assays by the Aliquot-part Method

Exercise 89

Object. Assay of Ipecac for Ether-soluble Alkaloids.

Materials Required. 10 Gm. of ipecac in No. 60 powder.
2 cc. of ether.

Procedure. 1. "Place 10 Gm. of Ipecac, in fine powder, in a dry, 250-cc. flask. Add 100 cc. of peroxide-free ether, measured at 25°, stopper the flask tightly, shake the mixture thoroughly, and allow it to stand for 5 minutes. Then add 10 cc. of ammonia T.S., stopper the flask tightly, shake it for 1 hour in a mechanical shaker, or intermittently during 2 hours, and allow to stand over night at a temperature not exceeding 25°. Again shake the mixture intermittently during 30 minutes, and allow the drug to settle at 25°. Quickly transfer to a separator exactly 50 cc. of the clear, supernatant liquid, representing 5 Gm. of Ipecac, rinse the measuring vessel with a small volume of peroxide-free ether, and add the rinsing to the solution in the separator." (See General Procedure 3*a*, page 332.)

Ipecac contains a number of alkaloids, the most important of which are emetine, cephaeline, and psychotrine. These three alkaloids occur in varying proportions in different varieties of the root. Psychotrine constitutes a relatively small proportion of the total alkaloids and is not known to be of importance therapeutically. Consequently, this alkaloid is eliminated in the assay process, since it is practically insoluble in ether, while the liberated emetine and cephaeline, being soluble in ether, are determined. The ipecac must be finely powdered, since it is not penetrated readily by the solvent because of its high starch content.

The aliquot portion of the ethereal solution contains an amount of emetine and cephaeline equivalent to one-half the weight of sample taken.

2. "Completely extract the alkaloids from the ether with approximately 1.0*N* sulfuric acid, preferably using 15 cc. the first time, or sufficient to insure an acid reaction, and 10 cc. for each succeeding extraction, and filtering all extractions through the same filter into a second separator."

The sulfates of emetine and cephaeline are formed and dissolve in the aqueous solution.

3. "To the combined acid solutions add about an equal volume of peroxide-free ether, render the mixture alkaline with ammonia T.S., and completely extract with successive portions of peroxide-free ether. Filter each portion of the ether extract into a flask or beaker, and carefully evaporate the combined ether extracts on a steam bath until nearly but not quite dry. Add 5 cc. of peroxide-free ether and exactly 10 cc. of 0.1*N* sulfuric acid, and heat on a steam bath to effect complete solution of the alkaloid and to remove all of the ether. Cool, dilute with 15 cc. of water, and titrate the excess of acid with 0.1*N* sodium hydroxide, using methyl red T.S. as the indicator. Each cubic centimeter of 0.1*N* sulfuric acid is equivalent to 24.0 mg. of the ether-soluble alkaloids of Ipecac." (See General Procedures 5, 6, and 7, pages 336 to 338.)

The ammonia sets the emetine and cephaeline free, and the liberated alkaloids dissolve in the ether.

The residue consists almost entirely of emetine and cephaeline which dissolve in the standard acid solution, forming emetine and cephaeline sulfates, as illustrated by the following reaction for emetine:

$$2C_{29}H_{40}N_2O_4 + H_2SO_4 \longrightarrow (C_{29}H_{40}N_2O_4)_2.H_2SO_4$$

Each cubic centimeter of 0.1*N* H_2SO_4 consumed corresponds to 0.024 Gm. of the ether-soluble alkaloids of ipecac.

The Pharmacopoeia requires that ipecac contain not less than 2 per cent of ether-soluble alkaloids. Calculate the percentage of ether-soluble alkaloids contained in the sample assayed and compare the results with the official requirement.

Ipecac Fluidextract, U.S.P., is assayed for total ether-soluble alkaloids by procedures that involve the same principles as the assay of ipecac.

Questions and Problems

1. Why is ipecac assayed for its content of ether-soluble alkaloids?

2. Ascertain the molecular weights of emetine and cephaeline from the table, U.S.P., and show how the alkaloidal equivalent of each cubic centimeter of 0.1*N* H_2SO_4 is derived.

3. Would the error in the assay result be very considerable if the ether-soluble alkaloids of the ipecac consisted almost entirely of emetine?

4. Enumerate several possible sources of error that might be encountered in the assay of ipecac.

Exercise 90

Object. Assay of Areca.

Materials Required. 8 Gm. of areca.
80 cc. of ether.
10 Gm. of anhydrous sodium sulfate.
0.5 Gm. of talc.

Procedure. "Place 8 Gm. of Areca, in moderately coarse powder and accurately weighed, into a glass-stoppered flask, add 80 cc. of ether, shake well, add 4 cc. of ammonia T.S., and shake during 10 minutes."

The most important alkaloid in areca is arecoline. Several other alkaloids, *viz.*, arecaidine, guavacine, guavacoline, and arecolidine, also occur in the drug in small amounts. These alkaloids, which are largely present in the drug as salts, are liberated by the NH_3 in the ammonia T.S. The arecoline, guavacoline, and arecolidine, although soluble in both water and ether, dissolve in large part in the latter, the arecaidine and guavacine mainly in the alkaline aqueous liquid, because they contain a carboxy group and form water-soluble salts with alkalies. Since arecoline is the methyl ester of arecaidine, and guavacoline is the methyl ester of guavacine, the carboxy groups are blocked, and these alkaloids do not form salts with alkalies.

2. "Add 10 Gm. of anhydrous sodium sulfate and shake during 5 minutes. Allow to settle and quickly decant the ether into another glass-stoppered flask."

Since arecoline, guavacoline, and arecolidine are soluble in both water and ether, the water present as a result of the addition of ammonia T.S. is removed by shaking with anhydrous sodium sulfate. After this treatment, all the arecoline, guavacoline, and arecolidine are dissolved by the ether and the other alkaloids, arecaidine and guavacine, which are almost insoluble in organic solvents, remain with the sodium sulfate and other constituents insoluble in ether.

3. "Add 0.5 Gm. of purified talc to the decanted ether solution and shake during 3 minutes, then add 2.5 cc. of distilled water and shake during 3 minutes more. Allow to stand until clear, quickly decant 50 cc. of the ether, equivalent to 5 Gm. of Areca, and . . . . "

The decanted ether layer, which contains fat and suspended matter, is freed from fat and clarified by shaking it with talc and a small amount of water and allowing it to settle. Exactly 50 cc. of the clear supernatant ether solution is decanted, so that the aliquot will represent 5 Gm. of the drug.

4. "Evaporate off about two-thirds of the ether."

About two-thirds of the ether is distilled off to reduce the volume to about 15 cc. and thus facilitate the shaking out of the alkaloids. The arecoline, guavacoline, and arecolidine, which are soluble in all proportions in ether and water, form salts with the acid which are readily soluble in water but only slightly soluble in ether.

5. "Extract the remaining ether solution in a separator with 15 cc. of 0.02*N* sulfuric acid and then with 3 portions of distilled water using 5 cc. each time. The 15 cc. of 0.02*N* sulfuric acid may be added directly to the ether in the glass-stoppered flask and the flask shaken for 10 minutes, after which its contents are then transferred to the separator. The flask is then washed with the three 5-cc. portions of distilled water, each being passed successively through the separator. To the combined acid and washings, add methyl red T.S. and titrate the excess acid with 0.02*N* sodium hydroxide solution. Each cubic centimeter of 0.02*N* sulfuric acid is equivalent to 3.101 mg. of alkaloids calculated as arecoline."

The acid used is 0.02*N* H_2SO_4, and the amount consumed is determined by residual titrations with 0.02*N* NaOH. The arecoline equivalent is computed as follows:

$$\underbrace{2C_8H_{13}O_2N}_{2 \times 155.20} + \underbrace{H_2SO_4}_{98.08} \longrightarrow (C_8H_{13}O_2N)_2.H_2SO_4$$

Since 98.08 Gm. of H_2SO_4 is equivalent to 2 × 155.20 Gm. of arecoline, each cubic centimeter of 0.02*N* H_2SO_4 is equivalent to

$$\frac{2 \times 155.20}{2 \times 50 \times 1{,}000} = 0.003108 \text{ Gm. of arecoline}$$

Inasmuch as an aliquot equivalent to 5 Gm. of the drug was used for the assay, the percentage of alkaloids in the sample will be

$$\frac{\text{cc. of } 0.02N\ H_2SO_4 \times 0.003108}{5} \times 100$$

The National Formulary requires that areca contain not less than 0.35 per cent of ether-soluble alkaloids calculated as arecoline. Calculate the per cent of alkaloids contained in the sample assayed.

TABLE 53. DRUGS AND PREPARATIONS ASSAYED BY THE ALIQUOT-PART METHOD

Substance	Amount used, Gm. or cc	Assayed for	Official requirement, per cent
U.S.P.			
Ipecac	10	Ether-soluble alkaloids	(n.l.t.) 2
Ipecac fluidextract	10	Ether-soluble alkaloids	1.8 to 2.2*
N.F.			
Areca	8	Arecoline	(n.l.t.) 0.35
Cinchona	5	Total alkaloids	(n.l.t.) 5
Cinchona tincture, compound	50	Total alkaloids	0.4 to 0.5*
Hydrastis	10	Ether-soluble alkaloids	(n.l.t.) 2.5
Hydrastis fluidextract	10	Ether-soluble alkaloids	2.25 to 2.75*
Hydrastis tincture	50	Ether-soluble alkaloids	0.45 to 0.55*

* Per cent weight to volume.
(n.l.t.) = not less than.

Alkaloidal Assays by the Total-extraction Method

Exercise 91

Object. Assay of Hyoscyamus for Total Alkaloids.

Materials Required. 25 Gm. of hyoscyamus in No. 40 powder.
10 cc. of alcohol.
100 cc. of ether.
100 cc. of chloroform.

Procedure. 1. Place 25 Gm. of Hyoscyamus in moderately coarse powder in an extraction thimble, and insert the thimble in a Soxhlet, or similar extractor. Moisten the drug with a mixture of 3 cc. of stronger ammonia T.S., 10 cc. of alcohol, and 20 cc. of ether, and mix thoroughly. Macerate overnight, then extract with ether for not less than 3 hr. or until the alkaloids are completely extracted. The following alternative process may be used: Moisten 25 Gm. of Hyoscyamus in moderately coarse powder (if a powder finer than "moderately coarse" is used for the assay, washed sand or asbestos fibers may be used to facilitate extraction) with a mixture of 3 cc. of stronger ammonia T.S., 20 cc. of ether, and 10 cc. of chloroform, in a small percolator, the outlet of which has been packed with a pledget of purified cotton. Macerate overnight, pack it in the percolator, and extract by percolating slowly with a mixture of 3 volumes of ether and 1 volume of chloroform. Continue the percolation until the 3 or 4 cc. of percolate last passed, when evaporated to dryness and the residue dissolved in approximately 0.5*N* sulfuric acid and treated with mercuric iodide T.S., show only a faint turbidity. (See General Procedure 3*b* and *c*, pages 333 and 334.)

Hyoscyamine, the chief alkaloid of hyoscyamus, and small quantities of other alkaloids are set free from the acids with which they are combined in the crude drug by the ammonia, and dissolve in the organic solvent. The levorotatory hyoscyamine is largely converted into its racemic isomer, atropine, in the ammoniacal solution.

2. If the volume of liquid obtained by either the Soxhlet or percolation method of extraction is large, reduce it to a convenient volume by evaporating on a water bath. Transfer the liquid to a separator, rinse the container with one or more small volumes of the solvent, and add the rinsings to the separator. Completely remove the alkaloids from the immiscible solvent by extracting with successive portions of approximately 0.5*N* sulfuric acid (see page 336), filtering each portion drawn off.

The large volume of liquid obtained, especially by cold percolation, is concentrated to a small volume to secure a convenient amount. It should not be evaporated to dryness because the chlorophyll and other nonalkaloidal residue make it difficult to dissolve completely the alkaloids in the acid. The alkaloids hyoscyamine and atropine, being esters, undergo hydrolysis readily in aqueous acid and alkaline solution, especially when heated. Consequently, the evaporation of the organic solvent should be conducted at a low temperature, preferably not exceeding 40°, after the addition of the acid. The evaporation may be carried out advantageously in a vacuum desiccator under reduced pressure. Upon filtration, the nonalkaloidal residue consisting mostly of chlorophyll is retained on the filter. This residue is dissolved in chloroform, treated with acid, and evaporated to extract completely the alkaloids. This method of obtaining the alkaloids in acid solution from the organic solvent avoids the formation of the stable emulsions frequently obtained by the shaking-out process. Quite stable emulsions are particularly liable to be encountered in shaking out alkaloids in the assay of hyoscyamus, belladonna, and stramonium, owing to the presence of small amounts of saponins which act as emulsifying agents.

The sulfates of the alkaloids are formed and dissolved in the acid solution, leaving most of the coloring matter, etc., in the acid insoluble residue.

3. Render the combined acid solutions distinctly alkaline with ammonia T.S., and completely remove the alkaloids at once by extracting with successive portions of chloroform. (See General Procedure 5, page 336.)

The alkaloids are set free by the ammonia water and dissolve in the chloroform.

4. Evaporate the combined chloroform extractions to dryness on a water bath and then heat in a bath of boiling water for 15 min. Dissolve the residue in a small volume of chloroform, evaporate to dryness on a water bath, and heat in a bath of boiling water for 15 min.

The solution of the alkaloids in organic solvent is evaporated to drive off the solvent and ammonia. The residue is repeatedly dissolved, evaporated, and dried to drive off small amounts of volatile amines which are basic and which, unless removed, would neutralize some of the acid in the next step of the procedure, with subsequent high results.

5. Dissolve the resulting residue in a few cubic centimeters of chloroform, add 15 cc. of 0.02*N* sulfuric acid, remove the chloroform by evaporation, cool, and titrate the excess acid with 0.02*N* sodium hydroxide, using methyl red T.S. as the indicator. Each cubic centimeter of 0.02*N* acid is equivalent to 5.787 mg. of the alkaloids of Hyoscyamus.

The residual alkaloids, which consist chiefly of the isomers atropine and hyoscyamine, react with some of the sulfuric acid as follows:

$$\underset{289.36}{2C_{17}H_{23}O_3N} + \underset{98.08}{H_2SO_4} \longrightarrow (C_{17}H_{23}O_3N)_2.H_2SO_4$$

Since 98.08 Gm. of H_2SO_4 is equivalent to 2 × 289.36 Gm. of atropine or hyoscyamine, each cubic centimeter of 0.1*N* H_2SO_4 is equivalent to $\frac{2 \times 289.36}{2 \times 50 \times 1{,}000} = 0.005787$ Gm. of the alkaloids of hyoscyamus. If exactly 10 Gm. of the drug is used as the sample for assay, the percentage of alkaloids in the hyoscyamus may be calculated as follows: $\frac{\text{cc. of } 0.02N\ H_2SO_4 \times 0.005787}{10} \times 100$ or cc. of $0.1N\ H_2SO_4 \times 0.05787$ = percentage of alkaloids in the sample.

The N.F. requires that hyoscyamus contain (n.l.t.) 0.040 per cent of alkaloids. Calculate the percentage of alkaloids contained in the sample assayed and compare the results with the official requirements.

Belladonna leaf, belladonna root, and stramonium are assayed by the same method as hyoscyamus, with the exception that smaller samples are used because these drugs contain larger amounts of alkaloids.

Assay of Preparations of Hyoscyamus, Belladonna, and Stramonium

Tinctures. The tinctures of hyoscyamus, belladonna (leaf), and stramonium are assayed by the same method except that the amounts of the tinctures employed as samples are designated so that approximately 0.030 Gm. of the alkaloids will be obtained in the assay residue. The assay method is the same as that for hyoscyamus, except that the extraction of the alkaloids is omitted, since they are already in solution. The assay process is illustrated in the following exercise.

Exercise 92

Object. Assay of Belladonna Tincture.

Materials Required. 100 cc. of belladonna tincture.
About 10 cc. of diluted alcohol.
150 cc. of chloroform.

Procedure. Measure accurately 100 cc. of Belladonna Tincture and evaporate it at a temperature not exceeding 100°, to a volume of about 10 cc. Transfer the concentrated liquid to a separator containing 25 cc. of chloroform, rinse the container with small portions of diluted alcohol, and add the rinsings to the separator. Add 25 cc. of water, and render the mixture alkaline by the addition of ammonia T.S.; then completely extract the alkaloids with successive portions of chloroform. Completely remove the alkaloids from the immiscible solvent by extracting with successive portions of approximately $0.5N$ sulfuric acid (see page 336), filtering each portion drawn off. Render the combined acid solutions distinctly alkaline with ammonia T.S., and completely remove the alkaloids at once by extracting with successive portions of chloroform. Evaporate the combined chloroform extractions to dryness on a water bath, and heat in a bath of boiling water for 15 min. Dissolve the residue in a small volume of chloroform, evaporate to dryness on a water bath, and then heat in a bath of boiling water for 15 min. Dissolve the resulting residue in a few cubic centimeters of chloroform, add 15 cc. of $0.02N$ sulfuric acid, remove the chloroform by evaporation, cool, and titrate the excess acid with $0.02N$ sodium hydroxide, using methyl red T.S. as the indicator. Each cubic centimeter of $0.02N$ acid is equivalent to 5.787 mg. of the alkaloids of belladonna leaf.

Pilular and Powdered Extracts. The pilular and powdered extracts of hyoscyamus, belladonna, and stramonium are assayed by the same method with the exception that different amounts of the extracts are used. The following National Formulary procedure illustrates the methods:

Assay of Pilular Extract of Hyoscyamus. "Weigh accurately 5 Gm. of Pilular Hyoscyamus Extract and mix it with a suitable quantity of an absorbent. Transfer the mixture completely, with the aid of a few cubic centimeters of alcohol or ether, to an extraction thimble, insert the thimble in a Soxhlet or similar extractor, moisten with a mixture of 3 cc. of stronger ammonia T.S., 5 cc. of alcohol, and 10 cc. of ether, and mix thoroughly. Macerate the mixture over night, and extract it for not less than 3 hours or until completely extracted, using ether as the solvent. Transfer the liquid to a separator, rinse the container with 1 or more small volumes of the solvent, and add the rinsings to the separator. Completely remove the alkaloids from the immiscible solvents by extracting with successive portions of approximately $0.5N$ sulfuric acid, filtering each portion drawn off. Render the combined acid solutions distinctly alkaline with ammonia T.S., and completely remove the alkaloids at once by extracting with successive portions of chloroform. Evaporate the combined chloroform extractions to dryness on a water bath, and continue the heating for 15 minutes. Redissolve the residue in a small volume of chloroform, evaporate to dryness on a water bath, and continue the heating for 15 minutes. Repeat this treatment for the third time. Dissolve the resulting residue in a few cubic centimeters of chloroform, add 15 cc. of $0.02N$ sulfuric acid, remove the chloroform by evaporation, cool, and titrate the excess acid with $0.02N$ sodium hydroxide, using methyl red T.S. as the indicator. Each cubic centimeter of $0.02N$ acid is equivalent to 5.787 mg. of the alkaloids of hyoscyamus, calculated as hyoscyamine."

Fluidextracts. Belladonna Leaf, Belladonna Root, Hyoscyamus and Stramonium Fluidextracts of the National Formulary are assayed by a method similar to that for belladonna leaf, as indicated by the following procedure:

Procedure. "Evaporate 10 cc. of Belladonna Leaf Fluidextract, accurately measured, on a water bath until the alcohol is all removed. Transfer this extract to 20 cc. of chloroform in a separator, using about 15 cc. of water and 1 cc. of ammonia T.S. to complete the transfer. Shake the mixture vigorously for 1 minute, separate the chloroform layer, and complete the extraction of the alkaloids with successive portions of chloroform. From the combined chloroform extractions in a separator, extract the alkaloids completely by shaking with successive small portions of dilute sulfuric acid (about 1 in 100). Filter the acid solutions successively through a small filter or pledget of cotton into a separator. Add a slight excess of ammonia water, and completely extract the alkaloids from the aqueous layer by shaking with successive portions of chloroform. Evaporate the combined chloroform extractions to dryness on a water bath and keep the dry residue at this temperature for 15 minutes. Again

TABLE 51. ALKALOIDAL ASSAYS BY THE TOTAL EXTRACTION PROCESS

Substance	Amount used, Gm. or cc.	Official requirement, per cent
U.S.P.		
Belladonna extract, pilular	3	1.15 to 1.35
Belladonna extract, powdered	3	1.15 to 1.35
Belladonna leaf	10	(n.l.t.) 0.35
Belladonna tincture	100	0.027 to 0.033
N.F.		
Belladonna leaf fluidextract	10	0.27 to 0.33
Belladonna ointment	20	0.110 to 0.140
Belladonna plaster	10	0.25 to 0.30
Belladonna root	10	(n.l.t.) 0.45
Belladonna root fluidextract	10	0.405 to 0.495
Hyoscyamus	25	(n.l.t.) 0.04
Hyoscyamus extract, pilular	5	0.135 to 0.175
Hyoscyamus extract, powdered	5	0.135 to 0.175
Hyoscyamus fluidextract	25	0.035 to 0.045
Hyoscyamus tincture	250	0.0034 to 0.0046
Ipecac tincture	50	0.18 to 0.22
Stramonium	10	(n.l.t.) 0.25
Stramonium capsules	10	0.238 to 0.288
Stramonium extract, pilular	3	0.90 to 1.10
Stramonium extract, powdered	3	0.90 to 1.10
Stramonium fluidextract	10	0.22 to 0.28
Stramonium tincture	100	0.22 to 0.028

dissolve the residue in chloroform, evaporate to dryness on a water bath, and continue the heating for 15 minutes. Repeat this treatment for the third time. Dissolve the resulting residue in chloroform, add 15 cc. of $0.02N$ sulfuric acid, remove the chloroform by evaporation, and titrate the excess acid with $0.02N$ sodium hydroxide, using methyl red T.S. as the indicator. Each cubic centimeter of $0.02N$ sulfuric acid is equivalent to 5.787 mg. of the alkaloids of belladonna calculated as atropine."

Ointment of Belladonna and Belladonna Plaster. Belladonna ointment and Belladonna plaster, N.F., are assayed by special methods designed to separate the alkaloids from the ointment or plaster base. The principles involved are similar to those already considered.

Questions and Problems

1. What isomeric change does the chief alkaloid of belladonna undergo in the assay process?

2. The alkaloids of belladonna have the structure of esters. What type of decomposition might they undergo if allowed to stand in contact for a long time with a strong alkali?

3. How much $0.1015N$ H_2SO_4 will be required to form the sulfates of the alkaloids from 25 Gm. of hyoscyamus leaves that contain 0.070 per cent alkaloids?

Chapter 17

ALKALOIDAL ASSAYS BY SPECIAL METHODS

A number of the official alkaloidal drugs and their preparations cannot be assayed by any of the type methods previously discussed because of the nature of the drug or of the properties of the alkaloids to be determined. Drugs of this type are assayed by special methods.

The assays of opium and its preparations differ from those of the other alkaloidal drugs, because morphine exhibits exceptional solubility properties; *i.e.*, it is practically insoluble in most organic solvents.

Exercise 93

Object. Assay of Opium.

Materials Required. 6 Gm. of opium.
3 Gm. of calcium hydroxide.
1 Gm. of ammonium chloride.
100 cc. of ether.
25 cc. of water saturated with morphine.

Procedure. 1. Weigh accurately 6 Gm. of Opium which, if fresh, should be in very small pieces and, if dry, in a fine powder. Place the Opium in a mortar and triturate it with about 40 cc. of warm water for 15 minutes. Transfer the contents of the mortar completely with the acid of 30 cc. of water into a flask, stopper the flask, and shake it every 10 minutes, or continuously in a mechanical shaker during 1 hour. The extraction of the opium may also be made in the following manner: Place the comminuted opium as described above in a flask with 70 cc. of water, add a few glass beads, if desired, and shake every 10 minutes, or continuously in a mechanical shaker during 2 hours, and allow to stand over night, then on the following morning shake the flask for 30 minutes.

"Pour the contents of the flask as evenly as possible upon a wetted filter of 10 to 11 cm. diameter, and, when the liquid has drained off well, wash the residue with about 20 cc. of water, carefully dropped upon the edges of the filter and its contents. Transfer the residue on the filter as completely as possible into the flask with the aid of about 10 cc. of water, shake it thoroughly during

10 minutes, and filter through the original filter. Filtration and washing of the residue may be facilitated by the use of a centrifuge. When the liquid has drained off, wash the flask and residue with small portions of water until the washings are nearly colorless. (The total filtrate will usually measure 300 to 350 cc.)"

The alkaloids of opium, about twenty-five of which are known, occur in the drug as meconates and sulfates. When the opium is agitated and macerated with distilled water, most of the meconates and sulfates of the alkaloids are dissolved. Complete extraction of the alkaloids is obtained when the residual opium is treated with a second portion of distilled water. By rubbing to a smooth paste, any lumps are broken up, so that the water can come in contact with all the particles of the drug. The residue is washed with distilled water after each extraction to displace all the solvent absorbed by the opium.

2. Evaporate the filtrate and washings in a tared dish to about 30 Gm., and allow to cool. Now add 3 Gm. of calcium hydroxide, triturate for 15 minutes, then transfer the mixture completely to a tared flask with the aid of small portions of water. Add sufficient water to make 54 Gm. and mix well. Filter through a dry filter of 10 to 11 cm. diameter, collecting the filtrate in a dry cylinder or a small flask. During the filtration keep the funnel covered with a glass plate and its stem well within the neck of the receiving vessel.

The combined aqueous filtrates are evaporated on a water bath to a volume of about 30 cc., since the alkaloids that are quite stable at 100° might be decomposed if the evaporation were carried out over a direct flame. The purpose of concentrating the aqueous alkaloidal extractive is to reduce the volume to a convenient working quantity. The concentrated extract is made up to a weight of exactly 54 Gm., so that an aliquot portion representing a definite weight of opium may be taken. When the aqueous opium extract is triturated with calcium hydroxide, the alkaloids are liberated from their salts and the morphine, codeine, and narcotine are dissolved in the alkaline calcium hydroxide solution, most of the other alkaloids of opium remaining as insoluble matter along with the calcium meconate, calcium sulfate, resins, etc. The mixture may be transferred to a flask and shaken for 15 min. to allow the reactions in which the alkaloids are liberated to go to completion, the flask being stoppered to prevent evaporation, so that the volume of liquid will not change appreciably, since an aliquot portion of it is employed in the assay.

3. Place 34 Gm. of the filtrate, corresponding to 4 Gm. of Opium, in an Erlenmeyer flask of suitable capacity, add 2 cc. of alcohol and 15 cc. of ether, and, after shaking, add 1 Gm. of ammonium chloride. Stopper the flask, shaking it frequently during 10 minutes, and set aside over night at a temperature of 5° to 10°. Remove the stopper and brush any adhering crystals back into the flask.

Since the total aqueous extract containing the alkaloids weighed 54 Gm. after adding the lime, 54 − 3 = the weight of liquid, and an aliquot portion of two-thirds of this liquid or 34 Gm. contains an amount of the alkaloids corresponding to $\frac{2}{3}$ of 6 = 4 Gm. of opium.

Ammonia is liberated from ammonium chloride:

$$2NH_4Cl + Ca(OH)_2 \longrightarrow 2NH_4OH + CaCl_2$$

and morphine is precipitated, since it is insoluble in ammoniacal solution and in ether. The alcohol causes the morphine to separate out in the form of relatively large crystals; whereas, when alcohol is not used, the alkaloid comes down in the form of a fine powder. The crystals are more easy to collect and less rapidly soluble in ether than the powder. The mixture is allowed to stand in a cool place for 12 hr. to allow the larger crystals to grow at the expense of the smaller ones. The codeine and narcotine remain in solution in the ether.

4. Decant the ether layer through a small filter paper or through a sintered glass crucible, rinse the flask and contents with 15 cc. of ether, and pass these washings through the filter. Again wash the filter and precipitate with an additional 10 cc. of ether. When all of the ether has passed through the filter, pour the water layer on the filter without trying to remove the crystals from the flask. Wash the crystals in the flask and the contents of the filter with small portions of water saturated with morphine, using a total of 40 cc. of the morphinated water. Then add, dropwise, 1 cc. of cold water to displace the morphinated water.

The crystals are washed with ether to remove any traces of codeine or narcotine, since these alkaloids are insoluble in ammonia water but soluble in ether. Water saturated with morphine is used to wash the crystals to prevent solution of the crystalline morphine, which is very slightly soluble in water. This aqueous solution removes any ammonia present, as well as any other water-soluble impurity adhering to the surface of the crystals, such as gums, coloring matter, etc. The morphinated water must be displaced from the paper filter with distilled water to avoid including in the final result some of the morphine from the morphinated water.

5. To the flask containing the crystals add about 15 cc. of boiling reagent methanol, agitate to dissolve as much of the morphine as possible, and pour the boiling solution over the morphine on the filter, receiving the filtrate in a suitable dry flask or beaker. Repeat this treatment with boiling reagent methanol eight to ten times, using 5 to 7 cc. each time, until all the morphine has been dissolved. Cool the methanol solution, and add to it exactly 25 cc. of 0.1*N* sulfuric acid. Dilute with 75 cc. of water, and boil carefully, or evaporate on a steam bath, to a volume of about 50 cc. Cool, and titrate the excess of acid with 0.1*N* sodium hydroxide, using methyl red T.S. as the indicator. Each cubic centimeter of 0.1*N* sulfuric acid is equivalent to 28.53 mg. of anhydrous morphine.

The morphine crystals dissolve in the 0.1*N* sulfuric acid with the formation of morphine sulfate:

$$\underset{2 \times 285.33}{2C_{17}H_{19}O_3N} + \underset{98}{H_2SO_4} \longrightarrow (C_{17}H_{19}O_3N)_2.H_2SO_4$$

One thousand cubic centimeters of *N* sulfuric acid is equivalent therefore to 285.33 Gm. of anhydrous morphine, and each cubic centimeter of 0.1*N* sulfuric acid is equivalent to $\frac{285.33}{10 \times 1{,}000} = 0.02853$ Gm. of anhydrous morphine.

The Pharmacopoeia requires that opium contain not less than 9.5 per cent of anhydrous morphine. Calculate the percentage of anhydrous morphine found in the sample of opium assayed and compare the results with the official requirements.

Granulated opium and powdered opium are assayed in exactly the same manner as described above. The general procedure used in the assay of tincture of opium is also the same, except that 60 cc. of the tincture is evaporated to remove the alcohol, the residue extracted with distilled water, etc., as directed in steps 1 to 2 of the above assay procedure. Opium extract of the National Formulary is also assayed by this method, but in the first step of the procedure, a 3-Gm. sample of the extract is dissolved in water, and the solution is diluted to 54 Gm. after the addition of the lime, etc. Camphorated opium tincture is assayed by a special method.

Questions and Problems

1. What functions do the calcium oxide and ammonium chloride serve in the above assay?

2. One gram of morphine is soluble in about 7,632 cc. of ether at 25°. Look up the solubility of codeine in the U.S.P. and explain how ether may be used to separate the alkaloids.

3. Why is water saturated with morphine used to wash the crystallized morphine?

4. Morphine contains a phenolic hydroxyl group. Explain why it is appreciably soluble in alkaline solutions.

TABLE 55. OFFICIAL SUBSTANCES ASSAYED BY THE OPIUM ASSAY METHOD

Substance	Amount used, Gm. or cc.	Equivalent of 1 cc. of $0.1N$ H_2SO_4	Anhydrous morphine, official requirement, per cent
U.S.P.			
Opium	6	0.02853	(n.l.t.) 9.5
Opium, granulated	6	0.02853	10.0 to 10.5
Opium, powdered	6	0.02853	10.0 to 10.5
Opium tincture	60	0.02853	0.95 to 1.05 W/V
Opium tincture, camphorated	100	0.005707*	0.035 to 0.045 W/V
N.F.			
Opium extract	3	0.02853	19.5 to 20.5

* Equivalent of 1 cc. of $0.02N$ H_2SO_4.
(n.l.t.) = not less than.

Exercise 94

Object. Assay of Colchicum Seed.

Materials Required. 15 Gm. of colchicum seed in No. 60 powder.
10 cc. of solution of lead subacetate.
2 Gm. of sodium phosphate.
150 cc. of chloroform.
20 cc. of ether.

Procedure. 1. Weigh accurately 15 Gm. of Colchicum Seed, in moderately fine powder, and place it in a suitable flask. Add exactly 290 cc. of distilled water and exactly 10 cc. of lead subacetate T.S., and weigh the flask and contents. Digest the mixture at a temperature of from 60 to 70° for 3 hr. with frequent agitation. Cool, add distilled water to the flask to restore the original weight, and filter into a dry flask, rejecting the first 20 cc. of the filtrate.

Colchicine is the only medicinally important constituent of colchicum. Both the seed and corm, which are assayed by the same method, contain a considerable amount of starch, sugar, gum, tannin, resin, fatty oil, and coloring matter from which the alkaloid must be separated. The free alkaloid, colchicine, is miscible in all proportions with water, and it is extracted from the drug when the latter is digested with water. The digestion temperature should not exceed 70°, because the starch content of the drug, especially of the corm, is gelatinized at higher temperatures, and because colchicine is less soluble in hot than in cold water. The lead subacetate is added to prevent solution of the tannin, gums, resin, and coloring matter with which it forms water-insoluble compounds and to liberate the colchicine from acids with which it may be combined in the crude drug. The mixture is cooled, and distilled water is added to restore the original weight of the sample, water, and lead subacetate mixture, so that an aliquot portion, corresponding to a definite weight of the sample taken, may be used. Two hundred cubic centimeters of the liquid, representing the alkaloids contained in two-thirds of the weight of the sample, are filtered off.

2. To 200 cc. of the clear filtrate add, in small portions, sufficient sodium phosphate (about 2 Gm.) to precipitate the lead completely, shake the mixture frequently during 30 min., and filter into a dry flask, rejecting the first 20 cc. of the filtrate.

The sodium phosphate precipitates the excess lead subacetate from the solution as insoluble lead phosphate, leaving sodium acetate in solution. A second aliquot portion of 100 cc., corresponding to one-third of the total sample used, or 5 Gm. of colchicum is taken.

3. Extract the alkaloids completely from 100 cc. of the filtrate by shaking with successive portions of chloroform. Test for complete extraction with iodine T.S. Carefully evaporate the combined chloroform extracts in a tared

flask to about 1 cc. on a water bath, then add to the residue 5 cc. of alcohol, and again evaporate about 1 cc. Twice more evaporate the residue with 5 cc. of alcohol, and then dry at 105° for 16 hr., and weigh.

Colchicine is miscible in all proportions with chloroform, and this solvent extracts the alkaloid, which is very feebly basic, from neutral or acid solutions. The alkaloid forms an addition product of unknown constitution of the formula $C_{22}H_{25}O_6N.2CHCl_3$ with chloroform. The chloroform is driven off from this addition product when it is treated with successive portions of alcohol and evaporated to dryness at 100°. The residue, dried to constant weight at 105° and weighed accurately, consists chiefly of crude colchicine and colchiceine.

4. Add to the residue 1 cc. of chloroform, 5 cc. of 0.1*N* sulfuric acid, and 5 cc. of distilled water, and heat the mixture at 70° for about 10 min. to dissolve the alkaloid and to evaporate the chloroform completely. Filter the solution through a pledget of purified cotton or a small filter paper, and wash the flask and the filter with distilled water until the washings show not more than a faint turbidity with iodine T.S. Drain the filter by application of suction to remove as much water as possible, and discard the filtrate and washings. Dissolve any residue on the filter by washing it first with alcohol and then with ether. Collect the alcohol and ether filtrates in the flask in which the alkaloid was weighed, evaporate to dryness, and dry the residue for 16 hr. at 105° and weigh. Deduct this weight from the weight of the residue previously obtained: the difference represents the quantity of colchicine, $C_{22}H_{25}NO_6$, yielded by 5 Gm. of Colchicum Seed.

A dilute solution of sulfuric acid heated to 70° is used to dissolve the colchicine completely from the residue, since this alkaloid decomposes rapidly when heated to 100° with strong mineral acids. The addition of a few drops of chloroform facilitates the solution of the colchicine from the residue. The colchicine in acid solution is filtered off through cotton and removed from the filtration medium by washing with distilled water, leaving acid-insoluble impurities on the cotton and in the flask. The impurities are dissolved by successive treatment with alcohol, which is miscible with water, and with ether. The total residual impurities are dried to constant weight at the same temperature as the crude colchicine; the weight is deducted from the weight of the latter previously obtained, giving the weight of colchicine that dissolved in the acid solution; *e.g.*, if an aliquot portion corresponding to 5 Gm. of colchicum yields 0.025 Gm. of crude colchicine and the residual impurity is found to weigh 0.005 Gm., then $\frac{0.025 - 0.005}{5} \times 100 = 0.4$ per cent of colchicine contained in the sample.

Colchicum seed must contain 0.45 per cent of anhydrous colchicine to meet the official requirement. Calculate the percentage of colchicine contained in the sample assayed and compare the result with the official requirement.

TABLE 56. OFFICIAL SUBSTANCES ASSAYED BY THE SAME METHOD AS COLCHICUM

Substance	Amount used	Aliquot portion corresponds to	Colchicine, official requirement, per cent
N.F.			
Colchicum corm	15 Gm.	5 Gm.	(n.l.t.) 0.35
Colchicum corm fluidextract	15 cc.	5 cc.	0.30 to 0.40*
Colchicum corm tincture, strong	45 cc.	15 cc.	0.12 to 0.16*
Colchicum seed	15 Gm.	5 Gm.	(n.l.t.) 0.45
Colchicum seed fluidextract	15 cc.	5 cc.	0.4 to 0.5*
Colchicum seed tincture	150 cc.	50 cc.	0.04 to 0.05*

* Per cent weight to volume.
(n.l.t.) = not less than.

Colchicum corm is assayed in the same way as colchicum seed. The extract, fluidextract, and tincture are also assayed by the same method, but in the case of the fluidextract, 15 cc. is taken as the sample and 275 cc. of water is used to bring the volume of the liquid to 290 cc., so that when 10 cc. of lead subacetate is added, the total volume of the mixture will be 300 cc. In the assay of the tincture of colchicum seed, a volume of 150 cc. is taken, evaporated to 15 cc., and made up to 300 cc. by the addition of 275 cc. of distilled water and 10 cc. of lead subacetate solution. In the Strong colchicum corm tincture, N.F., 45 cc. is taken, since the alkaloidal content of the corm is about three times that of the seed.

Questions and Problems

1. What exceptional solubility properties are exhibited by colchicine?

2. Why is lead subacetate used in the extraction of the alkaloid from the crude drug?

3. Why should not the aqueous mixture be heated above 70° in the extraction of colchicine from the powdered drug?

4. What property of colchicine makes it impossible to determine this alkaloid accurately by volumetric methods?

5. Why should the crude colchicine not be heated above 70° with the 0.1*N* sulfuric acid?

Exercise 95

Object. Assay of Nux Vomica.

Materials Required. 5 Gm. of nux vomica in No. 60 powder.
150 cc. of ether-chloroform mixture.
100 cc. of chloroform.

Procedure. 1. "Place 15 Gm. of Nux Vomica, in coarse powder, in a flask or bottle, add 150 cc., measured at 25°, of a mixture of 3 volumes of ether and 1 volume of chloroform, tightly stopper the flask, shake the mixture, and allow it to stand for about 5 minutes. Then add 10 cc. of stronger ammonia T.S., stopper the flask tightly, shake frequently, but gently, during 1 hour, and allow the mixture to stand overnight at a temperature not over 25°. Again shake the flask gently for 15 minutes, and allow the liquids to separate at 25°."

The alkaloids of nux vomica, which consist principally of strychnine and brucine, are set free by the ammonia water and dissolve in the ether-chloroform mixture.

2. Then quickly transfer to a small beaker exactly 100 cc. of the liquid, representing 10 Gm. of Nux Vomica, rinse the measuring vessel with a little chloroform, and add the rinsings to the beaker. Add 10 cc. of dilute sulfuric acid (1 in 20) and remove all of the ether-chloroform mixture by gentle heating on a water bath. Filter the remaining water solution into a separator, washing the beaker and filter with small portions of water until the washings are neutral to litmus paper. Make just alkaline with ammonia T.S., and extract with successive portions of chloroform until extraction is complete. Filter each chloroform extract through filter paper moistened with chloroform.

The alkaloidal sulfates are formed and dissolve in the acid solution; fats, resins, coloring matter, etc., remain in the ether-chloroform layer.

3. Carefully evaporate the combined chloroform extracts to dryness on a water bath, dissolve the residue by warming with 15 cc. of approximately 3 per cent

sulfuric acid, cool to 25°, and add 3 cc. of a mixture of equal parts of nitric acid and a 5 per cent solution of sodium nitrite in distilled water. Thoroughly stir this mixture, and allow it to stand for exactly 10 minutes at room temperature. At the expiration of this period, pour the red solution at once into a separator containing 50 cc. of chloroform and 15 cc. of sodium hydroxide solution (1 in 10), and rinse the flask with distilled water, adding the rinsings to the separator. Add sufficient sodium hydroxide solution (1 in 10), to the contents of the separator to render it distinctly alkaline to litmus paper and then add a few cubic centimeters more of the sodium hydroxide solution.

The strychnine and brucine are converted to their sulfates by the sulfuric acid. When the nitric acid and sodium nitrite mixture is added, the brucine is oxidized to a nonalkaloidal, red-colored, quinonoid compound having phenolic properties. The strychnine is very stable to oxidizing agents and is not attacked in significant amounts during the short period of oxidation. The decomposition of the brucine is completed in 10 min. If the mixture were allowed to stand for a longer period, a small amount of the strychnine would be oxidized. Consequently, the mixture is neutralized with sodium hydroxide to stop the oxidation, and an excess of the alkali is added to liberate the strychnine. Some workers prefer to liberate the strychnine by the addition of alkali prior to the addition of the organic solvent, because the complete decomposition of the strychnine salts is difficult to accomplish in the presence of organic solvents.

4. "Shake the mixture gently for 10 minutes and allow the liquids to separate. Draw off the chloroform layer into another separator and repeat the extraction with additional portions of chloroform until the alkaloid is completely removed. Add 10 cc. of distilled water to the combined chloroform extract, shake the mixture gently, and add a small piece of red litmus paper. The litmus paper should indicate not more than a slight alkalinity. If the water, after shaking with the chloroform, is strongly alkaline, draw off the chloroform into another separator, and shake it with another 10 cc. of distilled water. Draw off the chloroform, passing it through a filter paper moistened with chloroform, into a container. Wash the filter paper with warm chloroform, and add these rinsings to the container. Now shake the combined aqueous extract with 5 cc. of chloroform and draw off this chloroform, passing it through the chloroform-moistened filter paper into the main chloroform solution."

The mixture is shaken for 10 min. to decompose completely the strychnine salts. The liberated strychnine dissolves in the chloroform, whereas the phenolic quinonoid oxidation product from the brucine remains in the aqueous alkaline layer. Sodium hydroxide rather than ammonium hydroxide is used as the alkali because ammonium hydroxide is not a sufficiently strong base to combine completely with the phenolic oxidation products and prevent their passing into the chloroform. The combined chloroform extractive is washed with water to remove any alkali or alkali derivative of the phenolic oxidation products. This aqueous wash liquid is then washed with chloroform to remove any trace of strychnine.

5. "Evaporate the combined chloroform extracts very carefully on a water bath nearly but not quite to dryness. Add to the moist residue 7 cc. of 0.1*N* sulfuric acid, accurately measured, follow with 30 cc. of distilled water, and heat the mixture on a water bath until the alkaloid is dissolved and the odor of chloroform is dissipated. Cool to room temperature, and titrate the excess acid with 0.02*N* sodium hydroxide, using 1 drop of methyl red T.S. as the indicator. Each cubic centimeter of 0.1*N* sulfuric acid is equivalent to 33.44 mg. of $C_{21}H_{22}O_2N_2$."

TABLE 57. ASSAYS OF NUX VOMICA AND NUX VOMICA PREPARATIONS

Substance	Amount used, Gm. or cc.	Official requirement, per cent
N.F.		
Nux vomica	15	Strychnine = (n.l.t.) 1.15
Nux vomica extract	1.5	Strychnine = 7.00 to 7.75
Nux vomica fluidextract	10	Strychnine = 1.05 to 1.25
Nux vomica tincture	100	Strychnine = 0.105 to 0.125

(n.l.t.) = not less than.

The chloroform should not be evaporated to dryness before adding the acid, since there is likely to be some loss of strychnine by decrepitation if this is done. The strychnine is converted into its sulfate quantitatively by the 0.1*N* H_2SO_4; the excess acid is then determined by titration with 0.02*N* NaOH. The equation for the reaction is:

$$\underset{334.40}{2C_{21}H_{22}O_2N_2} + H_2SO_4 \longrightarrow (C_{21}H_{22}O_2N_2)_2.H_2SO_4$$

The number of cubic centimeters of 0.1*N* H_2SO_4 found upon residual titration with 0.020*N* NaOH subtracted from the number of cubic centimeters of standard acid solution originally added gives the quantity of standard acid solution that combined with the alkaloids.

The N.F. requires that nux vomica yield (n.l.t.) 1.15 per cent of strychnine. Calculate the percentage of strychnine contained in the sample assayed and compare the result with the official requirement.

Questions and Problems

1. Why must care be used to prevent emulsification when shaking out the ether-chloroform mixture with acid solution?

2. Explain the chemical changes that the strychnine undergoes during the various steps of the assay process.

3. Why is the determination of the total alkaloids of nux vomica not a correct measure of the therapeutic value of the drug?

4. If the total alkaloids of nux vomica were known to consist of strychnine and brucine in the ratio 3:1, what would be the proper equivalent to use in their volumetric estimation?

Assay of Caffeine-containing Drugs. Caffeine is the chief alkaloidal constituent of a number of drugs used in different parts of the world for beverage purposes, such as tea, maté, coffee, guarana, and kola. In the determination of the caffeine content of the drugs, it should be borne in mind that caffeine differs in its properties from most other alkaloids in the following ways: (1) It is very feebly basic and cannot be determined by the volumetric methods. (2) The free alkaloid is appreciably soluble in warm water (1 Gm. is soluble in 5.5 cc. at 80°). (3) It can be shaken out of acid solution readily with chloroform. (4) It is not precipitated from solution by mercuric iodide. (5) It sublimes without decomposition at about 180°.

The assay of kola, as described in the N.F. VIII, is typical of the procedure used for drugs that contain caffeine.

Exercise 96

Object. Assay of Kola.

Materials Required. 12 Gm. of kola in No. 60 powder.
250 cc. of chloroform.

Procedure. 1. Place 12 Gm. of Kola, in fine powder and accurately weighed, into a suitable container, add 120 cc. of chloroform, allow the mixture to stand about 5 min., and then add 6 cc. of ammonia water and 6 cc. of distilled water. Shake the mixture continuously for 1 hr. or intermittently during 2 hr., and allow it to stand overnight. Again shake intermittently during 30 min. and separate 100 cc. of the clear chloroform solution.

That portion of the caffeine that occurs as a salt of tannic acid is liberated by the ammonia and is dissolved in the chloroform along with the caffeine that occurs in the free state.

2. Evaporate the chloroform, and treat the residue with 10 cc. of sulfuric acid (1 in 100) with the aid of gentle heat. Filter the cooled solution into a separator, and wash the dish and filter with successive small portions of distilled water until no test for alkaloid is obtained with iodine T.S. in a portion (1 cc.) of the filtrate after acidifying strongly with diluted sulfuric acid.

The aliquot portion of the chloroform solution is evaporated to dryness so that the alkaloid may be dissolved in acid. This end is very difficult to accomplish by shaking out the chloroform solution with acid, since chloroform removes caffeine from acid solution. The residue should not be dried at a temperature exceeding 100°, because caffeine sublimes at higher temperatures.

When the residue consisting of fat, coloring matter, etc., and free alkaloid is treated with dilute sulfuric acid, the alkaloidal sulfate dissolves in the aqueous solution, leaving the water-insoluble impurities. Caffeine is precipitated from an acidulated, but not from a neutral or alkaline aqueous, solution by iodine test solution.

3. Make the combined filtrates alkaline with ammonia T.S., and again extract the alkaloid completely with successive portions of chloroform. Evaporate the combined chloroform solutions nearly to dryness, add about 1 cc. of neutralized alcohol, and continue evaporation to dryness. Dry the residue to constant weight at 80°. The weight obtained represents the yield of $C_8H_{10}N_4O_2$ from 10 Gm. of kola.

The alkaloid liberated from its sulfate by the ammonia is dissolved in the chloroform. Caffeine is determined gravimetrically, since it is a very feeble base which does not give a distinct end point with any indicator upon titration.

Kola is required to contain (n.l.t.) 1 per cent of anhydrous caffeine.

Questions and Problems

1. Why is not the caffeine extracted from the chloroformic solution with dilute acid in the usual manner?

2. Could mercuric potassium iodide solution be used to test for the complete extraction of the caffeine from the acid solution?

3. Why should caffeine be dried at a low temperature?

4. Explain why warm water can be used to dissolve the free alkaloid from the chloroformic residue in the assay of the fluidextract of kola.

Assay of Capsules and Tablets for Alkaloidal Content. The official assays of capsules and tablets for alkaloids are conducted in much the same way as the assays of the crude drugs in which the alkaloids occur (see pages 331 to 354), except that the methods of preparing the samples for assay differ and the procedures for the purification of the alkaloids are less involved, as a rule.

The usual procedure followed in taking the aliquot for an assay, in the case of capsules and tablets, is to empty about twenty capsules or to powder about twenty tablets and then weigh accurately the amount to be used in the assay. In some products, however, the aliquot is taken after the initial extraction of the alkaloid has been effected, *e.g.*, aminophylline tablets. In the latter instance, a definite number of tablets are dissolved in distilled water and ammonia T.S., the solution is diluted with distilled water to a definite volume and filtered. An accurately measured aliquot of the filtrate, equivalent to about 0.3 Gm. of aminophylline, is then taken for assay.

Modifications of the usual methods of assay may be necessary for tablets and capsules when coated. The methods of obtaining the quantities of the drug contained in capsules and tablets to be used for assay and the procedures followed for isolating and purifying the alkaloids are illustrated in the following exercises.

Exercise 97

Object. Assay of Aminophylline Tablets.

Materials Required. 20 Aminophylline Tablets.
20 cc. of 0.1*N* Silver Nitrate.

Procedure. 1. Weigh not less than 20 Aminophylline Tablets and reduce them to a fine powder without appreciable loss. Transfer completely to a 200-cc. flask with the aid of a mixture of 50 cc. of water and 15 cc. of ammonia T.S., and allow to stand for 30 minutes with frequent shaking. Dilute to 200 cc. with water, mix well, and filter through a dry filter into a dry flask, rejecting the first 20 cc. of the filtrate. Transfer an accurately measured aliquot, equivalent to about 300 mg. of aminophylline, to a 250-cc. Erlenmeyer flask and add water if necessary to make about 40 cc.

If the tablets are labeled to contain 3 grains each, twenty tablets, corresponding to about 4.0 Gm. of aminophylline, should be dissolved in a total volume of 200 cc. of distilled water and ammonia T.S. One cubic centimeter of the solution will then contain approximately 0.02 Gm. of aminophylline, and an aliquot of 155 cc. of the solution will contain about 0.3 Gm.

2. Add 8 cc. of ammonia T.S. and exactly 20 cc. of 0.1*N* silver nitrate, mix, and warm on the steam bath for 15 min. Cool the mixture to between 5 and 10° for 20 min., then filter, preferably through a filtering crucible under reduced pressure, and wash the precipitate three times with 10-cc. portions of water. Acidify the combined filtrate and washings with nitric acid, and add an excess of 3 cc. of the acid. Cool, add 2 cc. of ferric ammonium sulfate T.S., and titrate the excess silver nitrate with 0.1*N* ammonium thiocyanate. Each cubic centimeter of 0.1*N* silver nitrate is equivalent to 18.02 mg. of $C_7H_8N_4O_2$.

From the addition of the silver nitrate solution to the completion of the assay, the procedure is almost the same as that described under "Assay of Theophylline and Sodium Acetate." The explanations given under the latter are also applicable (see page 373).

Each cubic centimeter of 0.1*N* silver nitrate is equivalent to 0.01803 Gm. of anhydrous theophylline. Calculate the percentage of the labeled amount of anhydrous theophylline in the tablets assayed.

Exercise 98

Object. Assay of Strychnine Sulfate Tablets.

Materials Required. 50 strychnine sulfate tablets.
150 cc. of chloroform.
About 30 cc. of dehydrated alcohol.
5 cc. of neutral alcohol.

Procedure. 1. Weigh a counted number of not less than 20 Strychnine Sulfate Tablets, and reduce them to a fine powder without appreciable loss. Weigh accurately a portion of the powder, equivalent to about 30 mg. of strychnine sulfate, and transfer it to a 100-cc. volumetric flask. Add 10 cc. of water and 50 cc. of diluted sulfuric acid, shake the mixture occasionally during 2 hr., and allow to stand over night or for at least 12 hr.

The strychnine sulfate tablets generally available on the market contain $\frac{1}{100}$, $\frac{1}{60}$, $\frac{1}{50}$, $\frac{1}{40}$, or $\frac{1}{30}$ grain of the alkaloidal salt. Since an aliquot of the powdered tablets equivalent to not less than 0.13 Gm. of strychnine sulfate is directed to be taken for assay, fifty $\frac{1}{30}$-grain tablets or about one hundred sixty $\frac{1}{100}$-grain tablets will be required. An unusually large amount of diluted sulfuric acid is used in effecting the solution of the tablets to ensure an acid reaction in the presence of the binders, fillers, etc., used in making the tablets.

2. Add sufficient water to make the mixture measure 100 cc. (or 200 cc., if necessary, to effect solution), mix it thoroughly, and filter. Transfer a portion of the filtrate, equivalent to about 60 mg. of strychnine sulfate, to a separator, make the solution distinctly alkaline with ammonia T.S., and completely extract the strychnine with small successive 20-cc. portions of chloroform (at least six extractions will usually be required). Combine the chloroform extracts, and for each 50 cc. of the chloroform extract add 10 cc. of dehydrated alcohol, and evaporate the mixture nearly to dryness on a water bath; then add 5 cc. of neutralized alcohol and 25 cc. of $0.02N$ sulfuric acid, and warm on a water bath until the odor of chloroform is no longer perceptible.

Alcohol is added to the acid solution in the final heating to facilitate the removal of any remaining chloroform.

3. Titrate the excess of acid with $0.02N$ sodium hydroxide, using methyl red T.S. as the indicator. Each cubic centimeter of $0.02N$ sulfuric acid is equivalent to 8.57 mg. of $(C_{21}H_{22}N_2O_2)_2.H_2SO_4.5H_2O$.

One cubic centimeter of $0.02N$ sulfuric acid is the equivalent of 8.57 mg. of strychnine sulfate:

$$\underset{2 \times 334.40}{2C_{21}H_{22}N_2O_2} + \underset{98.08}{H_2SO_4} + \underset{90.10}{5H_2O} = \underset{856.96}{(C_{21}H_{22}N_2O_2)_2.H_2SO_4.5H_2O}$$

$$\frac{856.96}{2 \times 50 \times 1000} = 0.0085696, \text{ or } 8.57 \text{ mg. of strychnine sulfate.}$$

Calculate the percentage of the labeled amount of strychnine sulfate in the tablets assayed.

Questions and Problems

1. What are the official requirements for the alkaloidal contents of theobromine and sodium acetate tablets, ergonovine maleate tablets? How many tablets of each will be required for the assays?

Table 58. Official Capsules and Tablets Assayed for Alkaloids

Capsules or tablets	Weight of sample in aliquot, Gm.	Official requirement, per cent of labeled amount
U.S.P.		
Aminophylline	0.3	$C_{16}H_{24}N_{10}O_4.2H_2O$ = 73 to 84
Apomorphine hydrochloride	0.05	$C_{17}H_{17}O_2N.HCl.\frac{1}{2}H_2O$ = 90 to 110
Atropine sulfate (20 mg. or more)	0.06	$(C_{17}H_{23}O_3N)_2.H_2SO_4.H_2O$ = 93 to 107
Atropine sulfate (less than 20 mg.)	0.04*	$(C_{17}H_{23}O_3N)_2.H_2SO_4.H_2O$ = 90 to 110
Codeine phosphate	0.15	$C_{18}H_{21}O_3N.H_3PO_4.1\frac{1}{2}H_2O$ = 93 to 107
Codeine sulfate	0.15	$(C_{18}H_{21}O_3N)_2.H_2SO_4.5H_2O$ = 93 to 107
Colchicine	0.05	$C_{22}H_{25}NO_6$ = 90 to 110
Dihydromorphinone hydrochloride	0.07	$C_{17}H_{19}O_3N.HCl$ = 90 to 110
Ephedrine hydrochloride	0.25	$C_{10}H_{15}NO.HCl$ = 91 to 109
Ephedrine sulfate	0.10	$(C_{10}H_{15}NO)_2.H_2SO_4$ = 93 to 107
Ephedrine sulfate†	0.30	$(C_{10}H_{15}NO)_2.H_2SO_4$ = 91 to 109
Ergonovine maleate	0.00666	$C_{19}H_{23}N_3O_2.C_4H_4O_4$ = 90 to 110
Ergotamine tartrate	0.03	$(C_{33}H_{35}N_5O_5)_2.C_4H_6O_6$ = 90 to 110
Morphine sulfate	0.12	$(C_{17}H_{19}O_3N)_2.H_2SO_4.5H_2O$ = 93 to 107
Neostigmine bromide	0.30	$C_{12}H_{19}BrN_2O_2$ = 93 to 107
Quinidine sulfate	0.40	$(C_{20}H_{24}N_2O_2)_2.H_2SO_4.2H_2O$ = 94 to 106
Quinine sulfate	0.40	$(C_{20}H_{24}N_2O_2)_2.H_2SO_4.2H_2O$ = 94 to 106
Quinine sulfate†	0.40	$(C_{20}H_{24}N_2O_2)_2.H_2SO_4.2H_2O$ = 94 to 106
Scopolamine hydrochloride	0.05	$C_{17}H_{21}NO_4.HBr.3H_2O$ = 90 to 110
Theobromine and sodium acetate†	10.00	$C_7H_8N_4O_2$ = 53 to 67
Theophylline	0.25	$C_7H_8N_4O_2.H_2O$ = 94 to 106
N.F.		
Arecoline hydrobromide	0.30	$C_8H_{13}O_2N.HBr$ = 91 to 109
Caffeine and sodium benzoate	0.50	$C_8H_{10}O_2N_4$ = 43.5 to 53.5
Caffeine, citrated	0.50	$C_8H_{10}O_2N_4$ = 45 to 55
Cocaine hydrochloride	0.06	$C_{17}H_{21}O_4N.HCl$ = 91 to 109
Ephedrine sulfate and phenobarbital†	0.30	$(C_{10}H_{15}NO)_2.H_2SO_4$ = 91 to 109
Morphine and atropine sulfates	0.13	$(C_{17}H_{19}O_3N)_2.H_2SO_4.5H_2O$‡ = 91 to 109
Procaine hydrochloride	0.20	$C_{13}H_{20}O_2N_2.HCl$ = 92.5 to 107.5
Stramonium†	10.0	Stramonium alkaloids = 0.238 to 0.288§
Strychnine nitrate (20 mg. or more)	0.13	$C_{21}H_{22}O_2N_2.HNO_3$ = 92.5 to 107.5
Strychnine nitrate (1.2 to 20 mg.)	0.13	$C_{21}H_{22}O_2N_2.HNO_3$ = 91 to 109
Strychnine nitrate (less than 1.2 mg.)	0.13	$C_{21}H_{22}O_2N_2.HNO_3$ = 88 to 112
Strychnine sulfate (20 mg. or more)	0.10	$(C_{21}H_{22}N_2O_2)_2.H_2SO_4.5H_2O$ = 93 to 107
Strychnine sulfate (less than 20 mg.)	0.10	$(C_{21}H_{22}N_2O_2)_2.H_2SO_4.5H_2O$ = 90 to 110
Theophylline and sodium acetate	0.35	$C_7H_8N_4O_2$ = 53 to 67
Totaquine	4.00	Anhydrous quinine = (n.l.t.) 9.5. Total anhydrous crystallizable cinchona alkaloids = 66 to 84

* If tablets are of very small grainage (otherwise use 0.06 Gm.).

† Capsules.

‡ Morphine sulfate.

§ Of labeled amount of stramonium.

(n.l.t.) = not less than.

2. Why are the procedures for the assay of tablets less involved than those for the assay of crude drugs?

3. In what assays for tablets, other than strychnine sulfate, are aliquots taken before and after the initial extraction of the alkaloid?

4. How many strychnine sulfate tablets will be required for an assay if they are labeled to contain (*a*) $^{1}/_{60}$ grain of strychnine sulfate; (*b*) $^{1}/_{40}$ grain?

5. Why is the equivalent of the sulfuric acid volumetric solution used in the assay of strychnine sulfate tablets given as strychnine sulfate plus $5H_2O$ instead of anhydrous strychnine sulfate?

Assay of Alkaloidal Salts. The determination of the amount of alkaloid present in an alkaloidal salt or in a simple preparation containing an alkaloid or an alkaloidal salt is generally a relatively simple process, since the alkaloid does not need to be freed from a number of organic impurities as is the case in the assay of crude drugs. The solvents used and the gravimetric or volumetric methods employed differ according to the characteristic properties of the alkaloid determined.

Exercise 99

Object. Assay of Citrated Caffeine.

Materials Required. 1 Gm. of citrated caffeine.
75 cc. of chloroform.

Procedure. "Accurately weigh about 1 Gm. of Citrated Caffeine, previously dried at 80° for 4 hours, and dissolve it in 10 cc. of hot water. Add 8 cc. of sodium hydroxide T.S., cool the solution, and shake it in a separator with three or more successive portions of 20 cc. each of chloroform to effect complete extraction of the caffeine. Filter the combined chloroform solutions through a small filter, previously moistened with chloroform, into a tared dish. Wash the stem of the separator, the filter, and the funnel with 10 cc. of hot chloroform, adding the washings to the dish, and evaporate the combined chloroform solutions on a water bath, adding 2 cc. of alcohol just before the last trace of chloroform is expelled. Complete the evaporation of the solvent, and dry the residue, consisting of $C_8H_{10}N_4O_2$, at 80° for 4 hours and weigh."

The citrated caffeine is dried before it is weighed to drive off moisture. Since caffeine is a very weak base and citric acid is feebly acidic, it is probable that the aqueous solution of citrated caffeine is dissociated almost completely into caffeine and citric acid. Citric acid is quite soluble in chloroform, but its sodium salt is insoluble in this solvent. The chloroform, therefore, dissolves the caffeine, leaving the sodium citrate in the aqueous solution.

Exercise 100

Object. Assay of Theophylline and Sodium Acetate for Theophylline.

Materials Required. 0.3 Gm. of theophylline and sodium acetate.

Procedure. 1. "Place about 300 mg. of Theophylline and Sodium Acetate, accurately weighed, in a 250-cc. Erlenmeyer flask, and 50 cc. of water and 8 cc. of ammonia T.S., and gently warm the mixture on a steam bath until complete solution is effected."

The theophylline and sodium acetate, which is a hydrated mixture of theophylline sodium and sodium acetate, dissolves completely in the warm aqueous liquid.

2. "Add exactly 20 cc. of 0.1*N* silver nitrate, mix, and continue to warm on the bath for 15 minutes. Filter through a filtering crucible under reduced pressure while still warm, and wash the precipitate three times with 10-cc. portions of water."

The silver nitrate converts the theophylline sodium ($C_7H_7N_4O_2Na$) into the silver derivative ($C_7H_7N_4O_2Ag$), which is precipitated from hot ammonia solution in crystalline form. The precipitate is collected on a filter and washed with successive portions of distilled water to remove completely any adhering silver nitrate solution.

3. "Acidify the combined filtrate and washings with nitric acid and add an excess of 3 cc. of the acid. Cool, add 2 cc. of ferric-ammonium sulfate T.S., and titrate the excess of silver nitrate with 0.1*N* ammonium thiocyanate. Each cubic centimeter of 0.1*N* silver nitrate is equivalent to 18.02 mg. of $C_7H_8N_4O_2$."

The excess of 0.1*N* silver nitrate in the filtrate is determined by titration with 0.1*N* ammonium thiocyanate. In acid solution, ammonium thiocyanate reacts with silver nitrate to form insoluble silver thiocyanate, which is white in color. After all the silver is precipitated, any excess of 0.1*N* ammonium thiocyanate will react with ferric ammonium sulfate to precipitate ferric thiocyanate, which is red in color (see page 143). Each cubic centimeter of 0.1*N* silver nitrate consumed corresponds to 18.02 mg. of anhydrous theophylline, $C_7H_8N_4O_2$.

$$\underbrace{C_7H_7N_4O_2Na}_{180.17 + 22.997} + \underbrace{AgNO_3}_{169.89} \longrightarrow \underbrace{C_7H_7N_4O_2Ag}_{180.17 + 107.88} + NaNO_3$$

$$\frac{180.17}{10 \times 1{,}000} = 0.01802 \text{ Gm. of anhydrous theophylline}$$

$$\frac{\text{cc. of } 0.1N \text{ AgNO}_3 \times 0.01802 \times 10}{3} \times 100 = \text{per cent of anhydrous theophylline in the sample.}$$

The N.F. requires that theophylline and sodium acetate yield not less than 55 per cent and not more than 65 per cent of anhydrous theophylline. Calculate the percentage of anhydrous theophylline in the sample assayed.

Exercise 101

Object. Assay of Ephedrine Sulfate.

Materials Required. 0.4 Gm. of ephedrine sulfate.
100 cc. of ether.
20 cc. of distilled water saturated with sodium chloride.

Procedure. 1. Weigh accurately about 400 mg. of Ephedrine Sulfate, previously dried at 105° for 3 hours, and transfer it to a separator containing 10 cc. of water saturated with sodium chloride. Add 5 cc. of $1N$ sodium hydroxide, and extract first with 20 cc. of ether, then with six successive 10-cc. portions of ether, collecting the ether extracts in a second separator.

Ephedrine is liberated by the action of the sodium hydroxide upon ephedrine sulfate. The free base is soluble in water (about 1 in 20); therefore, a saturated solution of sodium chloride, in which it is practically insoluble, is used for dissolving the salt. Ether is used instead of chloroform in extracting the ephedrine from the aqueous solution, because this alkaloid is a sufficiently strong base to decompose chloroform and form ephedrine hydrochloride. (See test for the identity of ephedrine, U.S.P.)

2. Wash the combined ether extracts with two successive 5-cc. portions of water saturated with sodium chloride, and transfer the water to another separator. Shake the wash water with 10 cc. of ether, add this ether to the ether extract in the second separator, and discard the wash water.

A saturated aqueous solution of sodium chloride instead of distilled water is used for washing the ether extracts for the reason given above.

TABLE 59. ASSAYS OF ALKALOIDS, ALKALOIDAL SALTS, AND SIMPLE PREPARATIONS

Substance	Amount used, Gm. or cc.	Official requirement, per cent
U.S.P.		
Aminophylline	0.25	$C_7H_8N_4O_2$* = 75 to 82
Aminophylline injection	0.30†	$C_{16}H_{24}N_{10}O_4.2H_2O$ = 73 to 83
Caffeine and sodium benzoate	1.00	$C_8H_{10}N_4O_2$ = 47 to 50
Caffeine and sodium benzoate, injection	0.40†	$C_8H_{10}N_4O_2$ = 45 to 52‡
Codeine phosphate	0.50	$C_{18}H_{21}O_3N$ = (n.l.t.) 70
Emetine hydrochloride injection	0.12†	$C_{29}H_{40}N_2O_4.2HCl$ = 84 to 94‡
Ephedrine, anhydrous	0.50	$C_{10}H_{15}NO$ = (n.l.t.) 98.5
Ephedrine, hydrated	0.50	$C_{10}H_{15}NO$ = (n.l.t.) 94
Ephedrine hydrochloride	0.30	$C_{10}H_{15}NO.HCl$ = (n.l.t.) 98
Ephedrine sulfate	0.40	$C_{10}H_{15}NO$ = 75.5 to 77.3 corresponding to $(C_{10}H_{15}NO)_2.H_2SO_4$ = (n.l.t.) 98
Ergonovine maleate	0.10	$C_{19}H_{23}N_3O_2.C_4H_4O_4$ = (n.l.t.) 98
Ergonovine maleate injection	0.002†	$C_{19}H_{23}N_3O_2.C_4H_4O_4$ = 90 to 110‡
Eucatropine hydrochloride	1.00	$C_{17}H_{25}O_3N.HCl$ = (n.l.t.) 97
Mercurophylline injection	0.20†	$C_7H_8N_4O_2.H_2O$ = 93 to 107‡
Mersalyl and theophylline injection	0.20†	$C_7H_8N_4O_2.H_2O$ = 93 to 107‡
Morphine injection	0.10†	Morphine salt = 93 to 107‡
Neostigmine bromide	0.35	$C_{12}H_{19}BrN_2O_2$ = (n.l.t.) 98
Neostigmine methylsulfate	0.35	$C_{13}H_{22}N_2O_6S$ = (n.l.t.) 98
Neostigmine methylsulfate injection	0.05†	$C_{13}H_{22}N_2O_6S$ = 90 to 110‡
Papaverine hydrochloride injection	0.12†	$C_{20}H_{21}NO_4.HCl$ = 95 to 105‡
Procaine hydrochloride and epinephrine injection	0.15†	$C_{13}H_{20}N_2O_2.HCl$ = 95 to 105‡
Procaine hydrochloride injection	0.2†	$C_{13}H_{20}O_2N_2.HCl$ = 95 to 105‡
Quinine and urethane injection	0.50†	$C_{20}H_{24}N_2O_2.HCl.2H_2O$ = 95 to 105‡
Tetracaine hydrochloride	0.30	$C_{15}H_{24}N_2O_2$ = 86.5 to 88.5 corresponding to $C_{15}H_{24}N_2O_2.HCl$ = (n.l.t.) 98.4
Theobromine and sodium acetate	2.00	$C_7H_8N_4O_2$ = 55 to 65
N.F.		
Caffeine and sodium salicylate	2.00	$C_8H_{10}O_2N_4$ = 48 to 52
Caffeine, citrated	1.00	$C_8H_{10}N_4O_2$ = 48 to 52
Ephedrine spray	10.00	$C_{10}H_{15}NO$ = 0.9 to 1.1
Ephedrine spray, compound	10.00	$C_{10}H_{15}NO$ = 0.9 to 1.1
Ephedrine sulfate injection	0.25†	$(C_{10}H_{15}NO)_2.H_2SO_4$ = 95 to 105
Ephedrine sulfate jelly	10.00	$C_{10}H_{15}NO$ = 0.65 to 0.85
Ephedrine sulfate solution	10.00	$C_{10}H_{15}NO$ = 2.1 to 2.4
Ethylhydrocupreine hydrochloride	0.5	$C_{21}H_{28}O_2N_2$ = (n.l.t.) 90
Eucaine hydrochloride	0.5	$C_{15}H_{21}O_2N.HCl$ = (n.l.t.) 99
Phenacaine hydrochloride	0.50	$C_{18}H_{22}N_2O_2$ = 87.5 to 90.5
Quinine and urea hydrochloride	0.5	$C_{20}H_{24}N_2O_2$ = 58 to 65
Quinine and urea hydrochloride injection	0.5†	$C_{20}H_{24}O_2N_2$ = 78 to 84‡
Quinine phosphate	0.5	$C_{20}H_{24}O_2N_2$ = 74 to 78
Strychnine phosphate	0.4	$C_{21}H_{22}N_2O_2$ = 70 to 73
Theobromine and sodium salicylate	2.0	$C_7H_8O_2N_4$ = (n.l.t.) 46.5
Theophylline and sodium acetate	0.30	$C_7H_8N_4O_2$ = 55 to 65
Totaquine	4.00	Anhydrous quinine = (n.l.t.) 10; total anhydrous crystallizable cinchona alkaloids = 70 to 80

* Theophylline (anhydrous or hydrate).

† Amount of equivalent sought.

‡ Per cent of labeled amount.

(n.l.t.) = not less than.

3. If preferred, the ether extraction may be made in a continuous extraction apparatus. Add to the ether solution exactly 20 cc. of 0.1*N* sulfuric acid, stir thoroughly, warm until the ether is expelled, cool, and titrate the excess acid with 0.02*N* sodium hydroxide, using methyl red T.S. as the indicator. Each cc. of 0.1*N* sulfuric acid is equivalent to 20.43 mg. of $C_{10}H_{15}NO.H_2SO_4$.

The ephedrine combines quantitatively with the acid in accordance with the following reaction:

$$\begin{array}{ll} 2C_{10}H_{15}ON + H_2SO_4 \longrightarrow (C_{10}H_{15}ON)_2.H_2SO_4 \\ 2 \times 165.23 \qquad 98.08 \end{array}$$

Since sulfuric acid has a hydrogen equivalent of two, 1 cc. of 0.1N solution is equivalent to $\frac{330.46}{2 \times 10 \times 1,000} = 0.01652$ Gm. of anhydrous ephedrine.

Chapter 18

OTHER OFFICIAL ASSAYS INVOLVING THE USE OF IMMISCIBLE SOLVENTS

Use is made of the immiscible solvent principle of extraction and purification in the proximate determination of the amount of active constituent contained in a number of nonalkaloidal drugs, such as jalap, cantharides, and oleoresin of aspidium, and in the assay of certain synthetic medicinal products, *e.g.*, the soluble barbitals, diphenylhydantoin sodium, etc.

Exercise 102

Object. Assay of Cantharides.

Materials Required. 15 Gm. of cantharides in No. 40 powder.
75 cc. of petroleum benzin.
100 cc. of benzene.
50 cc. of chloroform.
25 cc. of absolute alcohol.

Procedure. 1. "Place 15 Gm. of Cantharides, in moderately coarse powder, in a pressure bottle of not less than 250-cc. capacity, add 150 cc. of a mixture of benzene, 2 volumes, and petroleum benzin, one volume, and then add 2 cc. of hydrochloric acid. Stopper the bottle tightly, shake it well, and allow it to stand for about 10 hours. Now gradually warm the bottle and its contents to about 40°, and maintain it at approximately that temperature with frequent shaking during 3 hours, avoiding evaporation."

Cantharidin, $C_8H_{22}O:(CO)_2:O$, is the inner anhydride, lactone, of the dibasic cantharidic acid, $C_8H_{12}O(COOH)_2$. Both cantharidin and the alkali salts of cantharidic acid occur in the beetles. When the above mixture is acidulated, the cantharidin and the liberated cantharidic acid dissolve in the warm benzene–petroleum benzin mixture together with fats, coloring matter, and other inactive constituents. Although a Soxhlet apparatus can be used to advantage in this assay, the official directions are to extract the drug by maceration, the purpose being to adapt the method to the equipment available in most pharmaceutical laboratories. The maceration should be carried out in a heavy-walled bottle (a magnesium citrate bottle serves very well), since the volatile solvents generate considerable pressure when warmed to 40°.

2. "Cool the mixture, decant or filter off 100 cc. of the clear solution, and evaporate this rapidly in a tared beaker or wide-necked flask to a volume of about 5 cc. Add 5 cc. of chloroform to the residue and set it aside in a moderately warm place."

The evaporation of the volatile solvents should be performed as rapidly as possible, because cantharidin begins to sublime at 86° and appreciable amounts are driven off with the solvent during slow evaporation. When chloroform is added and the mixture is allowed to stand in a warm place, the cantharidin crystallizes out upon evaporation of the chloroformic solvent.

3. "When the solvent has all evaporated, add to the crystals 10 cc. of a mixture of equal volumes of dehydrated alcohol and petroleum benzine, which has previously been saturated with pure cantharidin, allow the mixture to stand during 15 minutes, and then decant the liquid through a pledget of purified cotton. Wash the crystals with successive portions of a saturated solution of cantharidin, similar to that directed above, until free from fat and coloring matter, and pass the washings through the same pledget of purified cotton."

Cantharidin is only slightly soluble in the purified petroleum benzin and dehydrated alcohol mixture. The use of a mixture of these solvents previously saturated with cantharidin makes it possible to remove the fat, coloring matter, etc., with which the crystals are contaminated, without dissolving any of the crystals. Successive 2- to 3-cc. portions of the above wash mixture serve most efficiently to remove the impurities adhering to the crystals.

4. "Then wash the cotton with a small quantity of warm chloroform to dissolve any adhering crystals, collecting the chloroform in the tared flask or beaker containing the washed crystals, evaporate the solvent with the aid of a current of air, dry the crystals at 60° for 30 minutes, and weigh. The resulting weight represents the amount of cantharidin obtained from 10 Gm. of Cantharides."

Negligible quantities of the cantharidin are volatilized when it is dried for ½ hr. at 60°. The residue, which consists of cantharidin and cantharidic acid in variable amounts, is considered to be cantharidin. The weight of the residue corresponds to the cantharidin contained in 10 Gm. of the sample assayed.

The N.F. requires that cantharides yield not less than 0.6 per cent of cantharidin when assayed as directed above. Calculate the percentage of cantharidin contained in the sample assayed and compare the results with the official requirement.

Questions and Problems

1. Why is hydrochloric acid added in the initial extraction of the cantharides?
2. Why should the residue be dried at a low temperature?
3. Why is the maceration conducted with a warm solvent in a pressure bottle?

Exercise 103

Object. Assay of Aspidium Oleoresin.

Materials Required. About 3 Gm. of aspidium oleoresin.
150 cc. of ether.
3 Gm. of barium hydroxide.

Procedure. 1. Warm the Aspidium Oleoresin on a water bath, and stir until it is thoroughly mixed. Transfer about 3 Gm. of it, accurately weighed, to a 250-cc. flask, and dissolve it in 40 cc. of ether. Add 75 cc. of a 3 per cent solution of barium hydroxide, and shake the mixture vigorously for 5 min. Transfer the mixture to a separator, allow the liquids to separate completely, and draw off and filter the barium hydroxide layer. Rinse the flask with two 25-cc. portions of a 3 per cent solution of barium hydroxide, transfer each rinsing separately to the separator, shake the mixture for 1 min., allow the liquids to separate completely, and draw off and filter the barium hydroxide layer.

Aspidium oleoresin usually deposits granular, crystalline filicic acid or filicin upon standing. It is warmed to render it more fluid, so that the precipitate may be uniformly distributed throughout the sample. The filmarone originally present in the crude drug, aspidium, is converted largely into filicic acid and its amorphous modification, filicin, during the preparation of the oleoresin. The barium hydroxide neutralizes the filmarone, filicic acid, and filicin, forming the water-soluble barium compounds which dissolve in the aqueous layer of the immiscible solvents. The resin, fat, coloring matter, etc., remain dissolved in the ethereal layer.

2. Transfer the combined filtered barium hydroxide solutions to a separator, render distinctly acid to litmus paper by the addition of hydrochloric acid, and extract with three successive portions of 30, 20, and 15 cc. of ether.

The filicic acid and filicin are liberated from their barium salts and dissolved in the ether, leaving barium chloride in the aqueous solution.

3. Filter the combined ether solutions, and wash the filter with ether. Evaporate the filtrate and washings, and dry the residue at 105° for 2 hr. The weight of the crude filicin so obtained is not less than 24 per cent the weight of Oleoresin taken for the assay.

The residue consists mostly of filicin and small amounts of filicic acid.

Calculate the percentage of crude filicin contained in the aspidium oleoresin assayed, and compare the result with the official requirements.

Questions and Problems

1. Why should the oleoresin be warmed and mixed well before the sample is taken?

2. Why is the sample of oleoresin dissolved in ether? What function does the ether serve?

3. Look up the assay method for aspidium in the U.S.P. and explain briefly how this crude drug is assayed.

Exercise 104

Object. Assay of Jalap.

Materials Required. 10 Gm. of jalap in No. 60 powder.
20 cc. of 1 per cent hydrochloric acid.
90 cc. of chloroform.
120 cc. of alcohol.

Procedure. 1. Place 10 Gm. of Jalap, in fine powder and accurately weighed, with about 60 cc. of a mixture of 9 volumes of alcohol and 1 volume of water, into a flask provided with a reflux tube or condenser, and digest the mixture on a water bath during 30 minutes. Transfer the warm mixture to a small percolator, allow it to drain, press the marc down gently, and percolate with the warm alcohol-water mixture until 100 cc. of percolate, when cooled, is obtained.

The jalap is digested and percolated with alcohol to extract the resin completely.

2. "Cool and pipette 20 cc. of this percolate into a separator, add 40 cc. of chloroform and 10 cc. of hydrochloric acid (about 1 in 100), and shake for 2 minutes. Allow the mixture to separate, draw off the chloroform layer, and extract the aqueous layer twice more, using 15 cc. of mixture of 2 volumes of chloroform and 1 volume of alcohol each time. Shake the combined chloroform solutions with 10 cc. of hydrochloric acid (1 in 100), again separate, and wash this acid liquid twice with 15-cc. portions of the chloroform-alcohol mixture."

The resin of jalap is insoluble in aqueous acid solution. It dissolves in the alcohol-chloroform layer, leaving nonresinous constituents in the aqueous layer.

3. Pass the combined chloroform extractions through filter paper moistened with the chloroform-alcohol mixture, wash the filter with the chloroform-alcohol mixture, and evaporate the filtrate to dryness; add 2 cc. of alcohol and again evaporate. Dry the residue at 105° for 2 hr. The weight obtained represents the yield of total resins from 2 Gm. of Jalap.

The lower aqueous layer, containing small amounts of sugars, gums, coloring matter, etc., is drawn off and discarded. The alcohol-chloroform solution when evaporated to constant weight leaves a residue that consists of practically pure resin of jalap.

Ipomoea and podophyllum are also assayed by analogous methods, since the solubility properties of the resins that they contain are similar to those of resin of jalap.

Exercise 105

Object. Assay of Barbital Tablets.

Materials Required. 20 barbital tablets.
10 cc. of a 2 per cent solution of NaOH saturated with NaCl.
About 50 cc. of ether.
About 75 cc. of chloroform.

Procedure. 1. Weigh a counted number of not less than 20 Barbital Tablets, and reduce them to a fine powder without appreciable loss. Weigh accurately a portion of the powder, equivalent to about 300 mg. of barbital, transfer it completely to a separator, and dissolve it as far as possible in 10 cc. of alkaline sodium chloride solution, made by saturating a solution of sodium hydroxide (1 in 50) with sodium chloride. If lubricants other than stearic acid or stearates are present, extract the solution with two 15-cc. portions of ether, and discard the ether.

The barbital reacts with the NaOH to form barbital sodium, which is readily soluble in the aqueous liquid. When the solution

TABLE 60. OFFICIAL NONALKALOIDAL SUBSTANCES ASSAYED BY METHODS EMPLOYING IMMISCIBLE SOLVENTS

Substance	Amount used, Gm. or cc.	Official requirement, per cent
U.S.P.		
Aminopyrine tablets	1.0*	Aminopyrine = 94 to 106
Aspidium	40	Crude filicin = (n.l.t.) 1.5
Aspidium oleoresin	3	Crude filicin = (n.l.t.) 24
Barbital sodium	0.5	Barbital sodium = (n.l.t.) 98.5
Barbital sodium tablets	0.3*	Barbital sodium = 94 to 100†
Barbital tablets	0.3*	Barbital = 94 to 100†
Diethylstilbestrol capsules	20‡	Diethylstilbestrol = 90 to 110†
Diethylstilbestrol injection	0.005*	Diethylstilbestrol = 90 to 110†
Diethylstilbestrol tablets	0.005*	Diethylstilbestrol = 90 to 110†
Diphenylhydantoin sodium	0.3	Diphenylhydantoin sodium = 98.5
Diphenylhydantoin sodium capsules	0.3*	Diphenylhydantoin sodium = 93 to 107†
Fluorescein sodium	0.5	Fluorescein sodium = (n.l.t.) 98.5
Menadione injection	0.025	Menadione = 90 to 120†
Menadione sodium bisulfite	0.3	Menadione sodium bisulfite = (n.l.t.) 94
Menadione sodium bisulfite injection	0.05*	Menadione = 90 to 110†
Pentobarbital sodium	0.5	Pentobarbital sodium = (n.l.t.) 98.5
Pentobarbital sodium capsules	0.3*	Pentobarbital sodium = 90 to 105†
Phenobarbital elixir	25	Phenobarbital = 0.37 to 0.43 *W/V*
Phenobarbital sodium	0.5	Phenobarbital sodium = (n.l.t.) 98.5
Phenobarbital sodium injection	0.350	Phenobarbital sodium = 90 to 105†
Phenobarbital sodium tablets	0.35*	Phenobarbital = 94 to 106†
Phenobarbital tablets	0.3*	Phenobarbital = 94 to 106
Phenol ointment	2	Phenol = 1.8 to 2.2
Picrotoxin injection	0.05*	Picrotoxin = 90 to 110†
Quinacrine hydrochloride tablets	0.25*	Quinacrine hydrochloride (2H_2O) = 95 to 100†
Saccharin sodium	0.5 to 0.6	Saccharin sodium = (n.l.t.) 98
Thiopental sodium	0.5	Thiopental sodium = (n.l.t.) 97
Thiopental sodium, sterile	0.5	Thiopental sodium = (n.l.t.) 91.7
N.F.		
Acetophenetidine and phenyl salicylate tablets	0.08*	Phenyl salicylate = 90 to 110†
Aminopyrine elixir	5	Aminopyrine = 3.7 to 43 *W/V*
Barbital elixir	10	Barbital = 3.2 to 3.8 *W/V*
Cantharides	15	Cantharidin = (n.l.t.) 0.6
Cresol solution, saponated	50	Cresol = 46 to 52 *V/V*
Ipomea	10	Total resin = (n.l.t.) 15
Jalap	10	Total resin = (n.l.t.) 9
Pentobarbital elixir	25	Pentobarbital = 0.33 to 0.4 *W/V*
Pentobarbital sodium tablets	0.3*	Pentobarbital sodium = 90 to 105†
Phenyl salicylate tablets	0.3*	Phenyl salicylate = 91 to 109†
Podophyllum	10	Podophyllum resin = (n.l.t.) 5
Pyrethrum	15	Total pyrethrins = (n.l.t.) 0.5

* Amount of ingredient sought.

† Per cent of labeled amount.

‡ (n.l.t.) 20 capsules.

(n.l.t.) = not less than.

is shaken with portions of ether, organic matter, such as petrolatum used as a lubricant in the preparation of the tablets, dissolves in the ether, but the barbital sodium, being insoluble in ether, remains in the aqueous layer.

2. Add 2 cc. of hydrochloric acid and 5 cc. of water, shake well, and completely extract the barbital with chloroform. Test for completeness of extraction by extracting with an additional 10-cc. portion of chloroform and evaporating the solvent: not more than 0.5 mg. of residue remains. Wash the combined chloroform extracts with two portions of water acidified with a drop of hydrochloric acid, extract the washings with two 5-cc. portions of chloroform, and add these to the chloroform extract. Filter the combined extracts through a pledget of cotton or other suitable filter into a tared beaker, and wash the separator and the filter with several small portions of chloroform. Evaporate the combined filtrate and washings on a steam bath with the aid of a current of air, dry the residue of barbital at 105° for 2 hr., cool, and weigh.

The hydrochloric acid liberates the barbital from the barbital sodium, and the liberated barbital is extracted upon shaking with successive portions of chloroform.

Questions and Problems

1. Calculate the amount of barbital in each of the tablets.
2. Does the amount of barbital found fall within the tolerance limits established for these tablets in the Pharmacopoeia?
3. Consult the U.S. Pharmacopoeia for the formulas of barbital and soluble barbital and write equations for the reactions that occur in the assay, using graphic formulas.

Chapter 19

ASSAY OF ENZYME-CONTAINING SUBSTANCES

No methods are known whereby the quantity of enzymes in a substance can be accurately determined. Consequently, the assay of enzyme-containing substances is based on observations of their power to produce changes in other substances. In other words, the activity of the enzymes is measured. Thus, pepsin is evaluated on the basis of the quantity of egg albumen that it will digest, and extract of malt is assayed for its power to convert starch into soluble carbohydrates under definite conditions. Any condition that tends to increase or decrease the activity of the enzymes manifests itself in the assay results as an increase or decrease in the quantity of enzymes present.

Numerous factors tend to influence the activity of enzymes. Some of the more important of these factors are as follows:

1. Practically all enzymes are destroyed when they are heated to 100°. Many of them are gradually inactivated at comparatively low temperatures (35 to 40°), and many of them undergo a slow loss of activity at room temperature and even at 0°.

2. Enzymes have an *optimum temperature* of action; *i.e.*, for each enzyme there is a definite range of temperature at which it exhibits its maximum activity under definite conditions. The optimum temperature for most enzymes lies between 30 and 45°.

3. The activity of the enzymes is affected by the mediums in which they react; *e.g.*, pepsin is most active in acid mediums, and pancreatin is most active in alkaline mediums.

4. The enzymes are inactivated by many chemical agents, such as alcohol, salts of the heavy metals, tannin, and phosphotungstic acid, that precipitate the enzymes from solution.

Because the activity of enzymes is influenced by so many factors, it is necessary to adhere strictly to the procedures outlined in the various assay processes in order to secure concordant results.

Exercise 106

Object. Assay of Pepsin.

Materials Required. 0.1 Gm. of pepsin.

0.1 Gm. of N.F. reference pepsin. The N.F. reference pepsin consists of a powdered pepsin, carefully selected, dried, and packaged and having a digestive power of 3,000 times its weight of egg albumen. It may be obtained from the Committee on National Formulary.

Normal hydrochloric acid.

A hen's egg.

A No. 40 sieve.

A conical measure.

Procedure. 1. "Mix 35 cc. of 1*N* hydrochloric acid with 385 cc. of distilled water. Dissolve 100 mg. of Pepsin in 150 cc. of this dilute acid. Likewise dissolve 100 mg. of Reference Pepsin in another 150-cc. portion of the dilute acid."

When 35 cc. of 1*N* HCl is diluted to 420 cc., the concentration of acid in the solution is reduced to approximately 0.3 per cent. This is about the normal acidity of the stomach. Investigation has shown that pepsin is most active as an albumen digestive in mediums containing this concentration of acid. Other strongly dissociated acids that supply the same hydrogen ion concentration as 0.3 per cent HCl might be used, since the digestive activity of the pepsin is directly related to the hydrogen ion concentration of the reaction mixture.

2. "Immerse one or more hen eggs in boiling water during 15 minutes. Cool them rapidly to room temperature by immersion in cold water; remove the shell and pellicle and all of the yolk and at once rub the albumen through a clean, dry, No. 40 sieve, rejecting the first portion that passes through the sieve. Place 10 Gm. of the succeeding well-mixed portion in each of 3 wide-mouth bottles of about 100-cc. capacity."

The egg is immersed in boiling water for 15 min. to coagulate thoroughly the albumen. The albumen is coagulated so that its digestion by the pepsin may be better observed. The coagulated albumen is passed through a sieve to break it into small particles, so that a greater surface area will be exposed to the digestive action of the pepsin enzymes. The first portion of the albumen that passes through the sieve is rejected, since it may contain dust particles and traces of metallic compounds from the materials of the sieve.

3. "Immediately add 35 cc. of the dilute acid at one time or in portions and, by suitable means, thoroughly disintegrate the particles of albumen. Place the bottles in a water bath at 52°. After the contents of the bottles have reached that temperature, add exactly 5 cc. of the acidified solution of Pepsin to one bottle, 4.30 cc. of the same solution and 0.70 cc. of the dilute acid to another bottle, and exactly 5 cc. of the acidified solution of Reference Pepsin to the third bottle. At once stopper the bottles securely, invert them 3 times, and maintain them at a temperature of 52° for 2 hours and 30 minutes, agitating the contents equally every 10 minutes by inverting the bottles once."

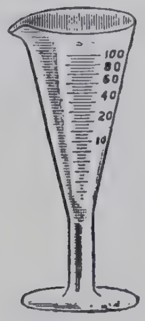

FIG. 27. Sedimentation cone.

The acid should be added at once, because the albumen loses moisture on exposure to the air and becomes tough and hard. The mixture is maintained at 52°, since pepsin exerts its optimum activity at this temperature. Exactly 5 cc. of the pepsin solution being assayed and of the solution of reference pepsin is added, because this quantity introduces 1 part of pepsin into the mixture to each 3,000 parts of albumen. Thus 0.1 Gm. of pepsin in 150 cc. gives $0.1:150::X:5$. $X = 0.003333$ Gm. pepsin in 5 cc. of the solution. $0.0033 \times 3{,}000 = 9.999$ (10 Gm.), the weight of albumen used.

4. "Remove the bottles from the bath, pour the contents into 100-cc. conically-shaped measuring vessels, having diameters not exceeding 1 cm. at the bottom and complying in other respects with the water and sediment tube ASTM Standard Method, D96-35, graduated from 0 to 0.5 cc. in 0.05-cc. graduations; from 0.5 to 3 cc. in 0.1-cc. graduations; from 3 to 5 cc. in 0.5-cc. graduations; from 5 to 10 cc. in 1-cc. graduations; from 10 to 25 cc. in 5-cc. graduations; and with graduation marks at 50-, 75-, and 100-cc. points. Transfer the undigested egg albumen which adheres to the sides of the bottles to the respective measuring vessels with the aid of small portions of distilled water until 50 cc. has been used for each. Mix the contents of each measuring vessel and allow them to stand for 30 minutes. The volume of the undissolved albumen in the measuring vessel corresponding to 5.0 cc. of the solution of Pepsin being assayed does not exceed the volume of the undissolved albumen in the measuring vessel corresponding to 5.0 cc. of the Reference Pepsin solution, and the volume of the undissolved albumen in the measuring vessel cor-

responding to 1.30 cc. of the solution of Pepsin being assayed, is not less than the volume of undissolved albumen in the measuring vessel corresponding to 5.0 cc. of the Reference Pepsin solution."

If the volume of egg albumen remaining undissolved from the sample containing 5.0 cc. of the solution of pepsin being assayed is not greater than that remaining from the sample containing the solution of reference pepsin, it is evident that the pepsin being assayed must be at least as active as the reference pepsin and that it must conform to the minimum official requirement. If the volume of egg albumen remaining undissolved from the sample containing 4.3 cc. of the solution of pepsin being assayed is not less than that remaining from the sample containing the reference pepsin, it is evident that the pepsin being assayed is a pepsin that will cause the solution of 3,500 times its weight of egg albumen. $4.3:5::3{,}000:X$. $X = 3{,}500$. The relative proteolytic power of pepsin, stronger or weaker than that just described, may be determined by ascertaining through repeated trials the quantity of the pepsin solution, made as directed in the assay, required to digest under the prescribed conditions 10 Gm. of boiled and disintegrated egg albumen.

Questions and Problems

1. Name several factors that affect the activity of pepsin.

2. Why is the proteolytic activity of pepsin determined in acid solution? What effect would the use of a neutral or alkaline medium have on the digestive power of pepsin?

3. Could proteins other than egg albumen be used to estimate the proteolytic activity of pepsin? Give examples.

4. What advantages does egg albumen possess as a source of protein for this assay?

Exercise 107

Object. Assay of Pancreatin for Starch Digestive Power.

Materials Required. 5 Gm. of powdered potato starch.
0.3 Gm. of pancreatin.
0.2 cc. of 0.1*N* iodine solution.

Procedure. 1. "Determine the percentage of moisture in U.S.P. Potato Starch Reference Standard by drying about 500 mg., accurately weighed, at 120° for 4 hours. Boil a sufficient amount of water for 10 minutes, and cool to room temperature. Use this water for all dilutions hereinafter specifying water. Thoroughly mix a quantity of the U.S.P. Potato Starch Reference

Standard, equivalent to 3.75 Gm. of dry Reference Standard, with 10 cc. of water. Add the mixture, with constant stirring, to 75 cc. of water, previously heated to about 55°, and contained in a tared, 250-cc. beaker. Rinse the remaining starch into the beaker with 10 cc. of water. Heat the mixture to boiling, and boil it gently, with constant stirring, for 5 minutes."

The percentage of moisture remaining in the starch is determined so that a sample equivalent to 3.75 Gm. of dry starch can be used for digestion. Thus, if the sample dried at 50° is found to contain 10 per cent of moisture, each gram of the sample is equivalent to 0.9 Gm. of dry starch. Then $3.75/0.9 = 4.1666$ Gm. of starch equivalent to 3.75 Gm. of dry starch.

The starch is mixed with cold water to obtain a paste, so that it will not form lumpy masses when the hot water is added. When this paste is added to warm water and the resulting mixture is boiled, the starch grains burst, liberating their contents, which dissolve in the hot water to form a more or less mucilaginous mass. Until the starch grains are broken down in this way, they remain insoluble and are not acted upon by pancreatin.

2. "Add enough water to make the mixture weigh 100 Gm., cool the paste to 40°, and place the beaker in a water bath maintained at 40°. Suspend 150 mg. of Pancreatin in 5 cc. of water in a 250-cc. beaker and add the suspension to the starch paste, mixing it well by pouring the mixture from beaker to beaker for 30 seconds, noting the time when the Pancreatin suspension was first added to the paste. Maintain the mixture at a temperature of 40° for exactly 5 minutes."

The water lost by evaporation is replaced so that a definite concentration of starch will be present during the digestion with the pancreatin. The solution of pancreatin must be prepared immediately before it is added to the starch solution, since amylopsin, the starch-splitting enzyme of pancreatin, becomes inactivated quite rapidly in aqueous solutions. The mixture is maintained at 40° for 5 min., because 40° is the optimum temperature for amylopsin. The regulation of the temperature is very important in this assay, since a variation of 1° throughout the period of digestion has been found to cause a difference of about 10 per cent in the activity of the amylopsin of pancreatin. The amylopsin converts the starch into soluble carbohydrates, chiefly maltose and isomaltose.

3. "Stir, and at once add 0.1 cc. of this mixture to a previously made mixture of 0.2 cc. of 0.1N iodine and 60 cc. of water, at a temperature of from 23° to 25°: no blue or violet color is produced."

The presence of starch and primary hydrolytic products of starch is indicated by the development of a blue, red, or violet color.

Pancreatin must be capable of converting at least twenty-five times its weight of starch into soluble carbohydrates in order to meet the pharmacopoeial requirements. Since 0.15 Gm. of pancreatin is employed to digest 3.75 Gm. of starch, each gram of pancreatin used corresponds to $3.75/0.15 = 25$ Gm. of starch. The relative amyloptic activity of a sample of pancreatin can be determined by repeated trials on varying quantities of starch.

Extract of malt of the N.F. is also assayed for its capacity to convert starch into soluble carbohydrates that do not give a color with iodine solution.

Questions and Problems

1. Define the terms *proteolytic enzyme* and *amylolytic enzyme.*

2. Why is the starch solution boiled before the digestion with pancreatin?

3. Look up the hydrolytic products of starch and explain what products might give a red or violet color with iodine.

Exercise 108

Object. Assay of Pancreatin for Casein Digestive Power.

Materials Required. 0.1 Gm. of casein.
1 cc. of 0.1N sodium hydroxide.
0.1 Gm. of pancreatin.
1 cc. of glacial acetic acid.
10 cc. of alcohol.

Procedure. 1. "Place 100 mg. of finely powdered casein in a 50-cc. volumetric flask, add 30 cc. of water, and shake well to bring the casein into suspension. Add exactly 1 cc. of 0.1N sodium hydroxide, and heat the mixture at 40° until the casein is completely dissolved, which should not require more than 30 minutes. Cool, add sufficient water to make 50 cc., and mix well."

Casein is a complex compound, or mixture of compounds, that yields amino acids upon hydrolysis. Consequently, it is soluble in alkalies. The casein is dissolved in alkali, in this case, because trypsin, the proteolytic enzyme contained in pancreatin, functions best in alkaline mediums. Casein that requires more than 30 min. to dissolve is usually of poor quality, containing an excess of fat.

2. "Dissolve 100 mg. of Pancreatin in 500 cc. of water. Mix 1 cc. of glacial acetic acid with 9 cc. of water and 10 cc. of alcohol. Place 5 cc. of the casein solution in a test tube, add to it 2 cc. of the well-shaken Pancreatin solution and 3 cc. of water, and mix by gentle agitation. Immediately immerse the test tube in a water bath at 40°, and keep it at this temperature for 1 hour. Then remove it from the bath, and add 3 drops of the acetic acid mixture: no precipitate is produced."

The concentrations of the solutions of casein and pancreatin are such that when they are mixed there will be 25 parts of casein present for each part of pancreatin. Thus, in 2 cc. of the pancreatin solution there is $2 \times \frac{0.1}{500} = 0.0004$ Gm. of pancreatin, and in 5 cc. of casein solution there is $5 \times \frac{0.1}{50} = 0.01$ Gm. of casein; $0.0004 \times 25 = 0.0100$.

The mixture is maintained at 40°, the optimum temperature for trypsin, for 1 hr. to allow the proteolytic action to proceed. The casein is digested by the pancreatic enzymes with the formation of soluble proteoses and possibly small amounts of amino acids. When the alcoholic acetic acid solution is added, the alkali is neutralized and any undigested casein is precipitated, since casein is insoluble in alcohol and in acetic acid. The proteoses and other products of casein digestion, being soluble in alcohol and acetic acid, are not precipitated. Consequently, if a precipitate is formed, it indicates that the proteolytic activity of the pancreatin is below standard; that is, that 1 part of the pancreatin is not capable of digesting 25 parts of casein.

Questions and Problems

1. Why is the protein digestive capacity of pancreatin determined in an alkaline medium?

2. Which of the enzymes of pancreatin take part in protein digestion?

3. Define the term *optimum temperature* as applied to enzymes.

4. How might the relative proteolytic power of a sample of pancreatin stronger or weaker than the official standard be determined?

Exercise 109

Object. Assay of Rennin.

Materials Required. 0.1 Gm. of rennin.

0.1 Gm. N.F. reference rennin (a carefully preserved, stable, powdered rennin that has been repeatedly tested for a number of years, so that its stability and its standard are definitely established. It coagulates approximately but not less than 25,000 times its weight of fresh cow's milk).

100 cc. of cow's milk.

Procedure. 1. "Mix 0.1 Gm. of Rennin with 50 cc. of distilled water by gentle stirring, and allow the mixture to stand exactly 15 minutes. Mix 0.1 Gm. of Reference Rennin [see above] with 50 cc. of distilled water by gentle stirring, and allow the mixture to stand for exactly 15 minutes."

When rennin is added to water, it is taken up slowly, forming a somewhat opalescent mixture. If shaken vigorously, the rennin tends to clump into large particles which separate out. When the mixture is stirred gently and allowed to stand, however, the rennin gradually absorbs water and dissolves.

2. "Place 50 cc. of well-mixed cow's milk in each of 2 glass vessels about 12 cm. high and 4.5 cm. in diameter. Warm the milk rapidly to a temperature of 43°, and maintain it on a water bath at this temperature. Add 1 cc. of the rennin solution to the milk in one vessel, and 1 cc. of the Reference Rennin solution to the milk in the other vessel, noting the time when each solution is added. Stir each milk mixture slowly for 10 seconds immediately after the solution is added. Note the time which elapses until the milk thickens, as shown by a distinctly convex surface when the vessel is tipped to an angle of about 45°."

The rennin solution is added to the milk, and the mixture is maintained at 43°, because rennin exhibits its optimum activity at that temperature. The mixture is stirred to obtain an equal distribution of the rennin throughout the milk. The thickening of the milk is caused by the conversion of the soluble casein in the milk into soluble paracasein and insoluble calcium paracasein.

3. "The rennin solution coagulates in not less than 90 per cent and not more than 110 per cent of the time required by the Reference Rennin solution."

"Note: Milk varies in its coagulability. Different lots of milk, or the same distributor's milk obtained on successive days, may be found to vary in coagulation with the Reference Rennin."

The time required for the coagulation of milk by rennin has been found to be inversely proportional to the amount of rennin present. This relationship may be formulated as follows:

$C \times T = K$, where C represents the concentration of rennin and T represents the time during which it acts on the milk and K is a constant. It is obvious that, when the amount of rennin used is reduced, the time required for the coagulation of a given amount of milk will be increased.

The National Formulary reference rennin is capable of coagulating twenty-five thousand (22,500 to 27,500) times its weight of fresh milk. In the assay 0.002 Gm. of rennin contained in 1 cc. is used to coagulate 50 cc. of milk. Therefore, 1 Gm. of rennin is used for each $50/0.002 = 25{,}000$ cc. of milk.

TABLE 61. OFFICIAL SUBSTANCES ASSAYED FOR ENZYME ACTIVITY

Substance	Substrate	Official requirement
U.S.P.		
Pancreatin	Potato starch	Converts (n.l.t.) 25 × its weight of starch into soluble carbohydrates.
	Casein	Converts (n.l.t.) 25 × its weight of casein into proteoses
N.F.		
Malt extract	Potato starch	Converts (n.l.t.) 5 × its weight of starch into water-soluble sugars.
Pepsin	Egg albumen	Digests 3,000 to 3,500 × its weight of egg albumen.
Pepsin and rennin elixir	Egg albumen	Proteolytic activity in each 100 cc. = (n.l.t.) 2.25 Gm. of reference pepsin.
Pepsin elixir	Egg albumen	Proteolytic activity in each 100 cc = (n.l.t.) 1.75 Gm. of reference pepsin.
Pepsin elixir, compound	Egg albumen	Proteolytic activity in each 100 cc. = (n.l.t.) 1.75 Gm. of reference pepsin.
Rennin	Milk	Possesses coagulating activity = 90 to 110 per cent of reference rennin.

(n.l.t.) = not less than.

The relative milk-coagulating power of rennin stronger or weaker than the specified strength may be determined by repeated trials, using larger or smaller quantities of rennin.

Questions and Problems

1. What substances tend to accelerate the activity of rennin?

2. If a 50-cc. sample of milk is found to have an acidity of 0.175 per cent, how much 1*N* NaOH solution would be required to reduce the acidity to 0.145 per cent?

3. Milk usually has a specific gravity of from 1.029 to 1.034. The National Formulary directs that rennin should be capable of coagulating twenty-five thousand times its weight of fresh milk. In the assay, however, a volume of 50 cc. of milk is employed for each 0.002 Gm. of rennin. If the milk used has a specific gravity of 1.030, what should be the weight-to-weight ratio of the milk coagulated to the rennin?

PART 3

PHYSICOCHEMICAL METHODS USED IN OFFICIAL PHARMACEUTICAL ANALYSIS

The analysis of many of the official substances for the purpose of identifying them and of determining their purity or therapeutic value is based on the measurement of certain physical and chemical characteristics or constants, considered in conjunction with the estimation of certain special components by assay, and qualitative tests for various substances commonly present as impurities or added for the purpose of adulteration.

The qualitative methods employed in the determination of the physical characteristics or physical constants of official substances are frequently applied to materials of widely divergent nature; *e.g.*, valuable criteria relative to the purity of volatile oils, sugars, and alkaloids may be gained by the measurement of the optical activity. The fundamental principles involved in ascertaining the physical constants of these substances are the same regardless of the nature of the substance, although slight modifications of the technique employed are frequently necessary.

The Pharmacopoeia and the National Formulary, as a rule, specify that certain methods and apparatus shall be employed in the determination of the physical constants of official substances. In such cases, the methods are known as *official methods*, and the apparatus as *official apparatus*.

Chapter 20

SPECIFIC GRAVITY AND DENSITY

The *absolute density* of a solid or liquid is the mass of unit volume of the substance. Generally, however, in using the term *density*, we refer to the *relative density* or the density at a given temperature relative to the density of distilled water, either at the same temperature, represented by d, or at 4°, represented by $d_{4^\circ}^{t^\circ}$. When, in the latter case, the density is corrected for the buoyancy of the air, the number obtained represents the *true specific gravity* of the substance. In common practice, however, and in all except very exact work, the specific gravity of a substance is taken as *the ratio of the weight of the substance to the weight of an equal volume of distilled water at the same temperature.*

In the official standards, the specific gravity of a substance has been established as *the ratio of the apparent weight of the substance in air at 25° to that of an equal volume of distilled water at the same temperature*, or $d_{25^\circ}^{25^\circ}$, except when otherwise stated and for alcohol, which should be determined at 15.56° and compared with water at the same temperature. The latter is the standard temperature established by the U.S. Revenue Department for the measurement of the specific gravity of alcohol.

A considerable number of methods have been developed by which the specific gravities of various substances can be determined. Only those methods of practical importance most commonly used in pharmaceutical analysis to establish the identity and purity of medicinal substances will be considered in the following pages. These methods may be classified as follows:

A. Methods used to determine the specific gravity of liquids.
  1. By the use of a pycnometer.
  2. By the use of the Westphal balance.

3. By weighing a solid of known specific gravity and weight in the liquid.
4. By the use of hydrometers.

B. Methods used to determine the specific gravity of solids, such as:

1. Solids heavier than and insoluble in water.
    - *a.* By weighing in air and then in water.
    - *b.* By determining the weight of water displaced.
2. Solids lighter than and insoluble in water.
    - *a.* By weighing immersed in water.
    - *b.* By flotation.
3. Solids heavier than and soluble in water.
    - *a.* By weighing in air and then in some liquid of known specific gravity and in which the solid substance is insoluble.
4. Solids lighter than and soluble in water.
    - *a.* By weighing immersed in some liquid of known specific gravity and in which the substance is insoluble.

METHODS USED TO DETERMINE THE SPECIFIC GRAVITY OF LIQUIDS

1. By the Use of a Pycnometer. The specific gravity of liquids can be determined very accurately by means of some form of pycnometer or specific-gravity bottle, such as that shown in Fig. 28. Although a number of different forms of pycnometers have been developed by investigators, those described may be used for prac-

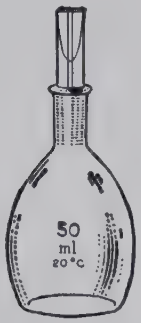

Fig. 28. Specific gravity bottle.

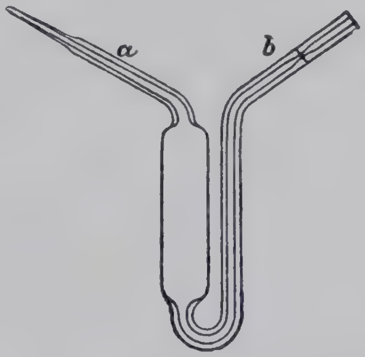

Fig. 29. Sprengel-Ostwald pycnometer (modified form).

tically all the determinations of the specific gravity of liquids required in pharmaceutical analysis.

The Sprengel-Ostwald Pycnometer. One of the simplest and most generally useful forms of pycnometers is that known as the Ostwald modification of the Sprengel tube (Fig. 29). This form of pycnometer, which can be easily made by the student, consists of a thin glass tube, 10 to 15 mm. in diameter, to which narrow tubes *a* and *b* are sealed and bent as shown in the figure. At about the middle of tube *b*, the bore should be slightly constricted and a graduation mark scratched or etched on the glass. Tube *a* should be drawn out to a point and fire polished, so that the bore is constricted. Some of the commercially available pycnometers of this type (Fig. 30) are provided with ground-on glass caps which fit over the ends of the tubes *A* and *B*. In all except very accurate work, however, these caps are unnecessary.

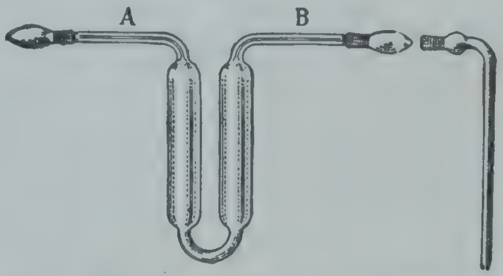

FIG. 30. Sprengel-Ostwald pycnometer with ground-on glass caps and ground-on glass side tube for use in filling.

The volume of the pycnometer should be about 5 to 15 cc., thus permitting an accuracy of about one unit in the fourth decimal place. When only a very small volume of liquid is available for a determination, however, a tube of small bore, bent in the shape indicated in Fig. 30, and of a capacity of 0.5 cc., may be used in approximate specific-gravity determinations.

In carrying out a determination with this type of pycnometer, it should be cleaned by washing successively with water, alcohol, and ether and then dried by drawing a current of warm air through the tube. The pycnometer, cleaned and dried, is first weighed empty. To do this, it is suspended from the end of a balance beam by means of a double hook made from either platinum or copper wire, preferably the former. The pycnometer is then filled with distilled water, which has been previously boiled to expel dissolved carbon dioxide and allowed to cool, by applying gentle suction through a rubber tube attached to the tube *b* while the tip of the tube *a* dips into the water. The introduction of air bubbles into the pycnometer must be carefully avoided. The filled pycnometer is then suspended in a beaker of water, along with a thermometer, and the temperature is kept constant at the desired level.

When the pycnometer and its contents have acquired the same temperature as the bath, usually in about 20 min., the amount of water must be so adjusted that it exactly fills the pycnometer from the point of the tube *a* to the graduation mark on *b*. If there is too much water in the pycnometer, a piece of filter paper is carefully touched to the tip of the tube *a*, thereby drawing water from the pycnometer until the meniscus corresponds to the mark on *b*. If the pycnometer is not completely filled, a rod or tube carrying a drop of water is touched against the end of the tube *a*, and water is drawn into the tube by capillarity.

The pycnometer is removed from the bath and the outside is dried with a cloth, using care to prevent the expulsion of water from the tube by expansion due to the heat of the hands, and weighed.

After the pycnometer filled with water has been weighed, it is emptied, dried once more, and filled with the liquid the specific gravity of which is to be determined. It is placed in the constant-temperature bath, the volume of liquid is adjusted to the mark, the pycnometer is dried with a cloth in the same manner as before and weighed.

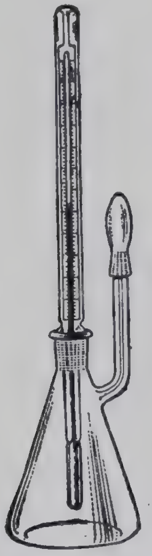

FIG. 31. Geissler pycnometer.

The Geissler pycnometer or specific-gravity bottle (Fig. 31) is a type of pycnometer well suited to accurate determinations and can be procured in sizes varying from 25- to 100-cc. capacity. Pycnometers of this type are provided with a thermometer stopper and a capillary overflow tube fitted with a ground-glass cap to prevent evaporation. The cap should have a very small perforation in its top to permit the liquid to expand without forcing the cap loose. The thermometers provided with these pycnometers are not always accurate and should be tested by comparison with one of known accuracy whenever exact determinations are made.

In ascertaining the specific gravity of a liquid with this pycnometer, the thermometer and cap are removed, the previously cleaned bottle is filled with the liquid, which has been cooled to from 1 to 3° below the temperature at which the specific gravity is to be determined. The thermometer is then fitted to the bottle, using due precautions, so that no air bubbles remain in the bottle. The pycnometer with its contents is warmed to the required temperature, any excess liquid that exudes from the capillary tube is carefully wiped off, the cap is fitted to the side tube, and the whole is weighed.

The Determination of the Specific Gravity of a Fat or Oil. The official procedure for the determination of the specific gravity of a fat or oil by the pycnometer method, which may be applied to most liquid substances is as follows:

> The specific gravity of a fat or oil shall be determined at 25°, except when the substance is a solid at that temperature. In the latter case the specific gravity shall be determined at the temperature directed in the respective monograph and referred to water at 25°.
>
> Clean a pycnometer (use a Sprengel or other suitable pycnometer with a well-fitted capillary stopper) by filling it with chromic acid cleansing mixture, and allowing it to stand for at least 4 hours. Empty the pycnometer, and rinse it thoroughly with water; then fill it with recently boiled water previously cooled to about 20°, and place in a constant temperature bath at 25°. At the end of 30 minutes adjust the level of the water to the proper point on the pycnometer; insert the perforated cap or stopper; remove from the bath, wipe dry with a clean cloth free from lint; and, after allowing to stand for 30 minutes, weigh. Empty the pycnometer, rinse several times with alcohol and then with ether, allow it to become perfectly dry, remove any ether vapor, and weigh. Ascertain the weight of the contained water at 25° by subtracting the weight of the pycnometer from its weight when full.
>
> Fill the clean, dry pycnometer with the oil at a temperature below that at which the determination is to be made; place it in a constant temperature bath at the specified temperature for 30 minutes; adjust the level of the oil to the proper point on the pycnometer; insert the cap or stopper, wipe dry; allow to stand for 30 minutes; and weigh. Subtract the weight of the empty pycnometer from its weight when filled with oil, and divide the difference by the weight of water contained at 25°. The quotient is the specific gravity at the temperature of observation, referred to water at 25°.

The Determination of Alcohol Content Using a Pycnometer. It is very difficult to effect a quantitative separation of alcohol from the solvents ordinarily employed in the manufacture of pharmaceutical preparations, but it is comparatively easy to prepare an aqueous distillate containing all the alcohol in the original mixture and to estimate the alcohol in the distillate by some physical means, such as the determination of the specific gravity, the index of refraction, or the boiling point of the solution. The specific-gravity method is official, and it is probably the most accurate and satisfactory of the methods mentioned above. This method consists of separating the alcohol from substances other than water contained in the preparation by distillation or other procedures and determining the per cent of alcohol in the preparation from the specific gravity of the distillate.

General Process. Measure accurately not less than 25 cc. of the liquid in which the alcohol is to be determined, and note its temperature. Transfer it to a suitable distilling apparatus and, if its alcohol content is thought to be not more than 30 per cent, dilute it with an equal volume of distilled water, using the water to rinse the vessel that was used for measuring unless this vessel is a graduated pipette which has been standardized on the basis of the amount delivered. Distill and collect a volume of distillate of about 2 cc. less than the volume of the original test liquid, adjust to the temperature at which the original test liquid was measured, add sufficient water to measure exactly the original volume of the test liquid, and mix thoroughly. Determine the specific gravity of the liquid at 25°, and from this result ascertain the percentage, by volume, of alcohol contained therein by consulting alcoholometric tables (see U.S.P.). The proportion of alcohol, by volume, in the distillate equals that in the liquid examined.

If the liquid under examination contains more than 30 per cent of alcohol, proceed as directed above, except: dilute the sample with about twice its volume of distilled water and collect a volume of distillate of about 2 cc. less than twice the volume of the original test liquid, bring to the temperature at which the original liquid was measured, add sufficient distilled water to measure exactly twice the original volume of the test liquid, and determine the specific gravity. The proportion of alcohol by volume in this distillate, as ascertained from its specific gravity, equals one-half that in the liquid examined.

The distillate must be clear or only slightly cloudy and must not contain more than traces of volatile substances other than alcohol and water.

This general method without modification is suitable for examining most fluidextracts and tinctures, provided the capacity of the distilling flask is sufficient (commonly two to four times the volume of the liquid to be distilled) and the rate of distillation is such that clear distillates are produced. Some alcoholic preparations will, however, require special treatment or the observance of special precautions to yield suitable distillates. If distillates are cloudy, they may be clarified by agitation with talc, or with precipitated calcium carbonate, and filtered, after which the temperature of the filtrate is adjusted and the alcohol determined from the specific gravity. All this should be done under conditions that will minimize the loss of alcohol by evaporation.

Frothing. Liquids that froth to a troublesome extent during distillation may be distilled by strongly acidifying them with phosphoric or sulfuric acid, or by the addition of a slight excess of calcium chloride solution or a little paraffin or yellow wax to the distilling flask.

Bumping. Liquids that tend to bump badly when heated (particularly resinous solutions) may be distilled by making them alkaline with magnesia magma or by placing pieces of pumice, glass beads, or similar materials in the distilling flask with the liquid, or by similar means of distributing the heat.

Glycerin. Liquids that contain glycerin must be diluted with sufficient distilled water so that the residue, after distillation, will contain at least 50 per cent of water.

Iodine. All Solutions of Iodine must be deprived of free iodine, before being distilled, by treatment with powdered zinc or by decoloration with just sufficient solution of sodium thiosulfate, followed by a few drops of sodium hydroxide T.S. to fix volatile sulfur compounds.

Volatile Substances. Spirits, elixirs, tinctures, etc., that contain appreciable proportions of volatile materials other than alcohol and water, such as volatile oils, chloroform, ether, camphor, etc., are treated as follows: Mix the accurately measured liquid with about an equal volume of saturated solution of sodium chloride in a separator, then add a volume of petroleum benzin equal to the sample, and shake the mixture to extract the interfering volatile ingredients. Draw off the separated lower layer and extract the benzin solution with two successive portions of a saturated solution of sodium chloride, using about one-half as much each time as was used in the first extraction mixture. Combine the aqueous saline solutions and distill the mixture in the usual way, collecting a volume of distillate having a simple ratio to the volume of the original liquid.

A troublesome emulsion may develop in the liquid mixture when it is shaken with the benzin. In this case, dilute a fresh portion of the original liquid with water and distill it as directed in the general process. Then treat this distillate as directed above, using purified petroleum benzin and sodium chloride solution, and distill the saline solution so produced, to obtain a distillate that is free from volatile substances other than alcohol and water.

In preparing *collodion* for distillation, water is used in place of the saturated solution of sodium chloride directed above.

If volatile oils are present in small proportions only, and a cloudy distillate is obtained, the benzin treatment not having been employed, the distillate may be clarified and rendered suitable for the specific gravity determination by shaking it with about one-fifth its volume of petroleum benzin, or by filtering it through a thin layer of talc.

Other Preparations Requiring Special Treatment. Preparations containing free ammonia, as aromatic spirit of ammonia, must be rendered slightly acid with sulfuric acid before being distilled.

Ethyl nitrite spirit and glyceryl trinitrate spirit must be treated with a small excess of sodium hydroxide before being distilled. The use of this expedient in examining ethyl nitrite spirit necessitates that a suitable correction be subtracted from the apparent result of the alcohol determination to compensate for the alcohol produced during the decomposition of the ethyl nitrite.

Chloroform liniment, camphor and soap liniment, and soft soap liniment are treated with an excess of sulfuric acid to effect decomposition of the soap before they are extracted with purified petroleum benzin as directed in the general process.

Exercise 110

Object. To Determine the Alcohol Content of Belladonna Leaf Fluidextract.

Materials Required. 25 cc. of belladonna leaf fluidextract.
A distilling apparatus.
A flask graduated to hold 50 cc.
A pycnometer.

Procedure. 1. Place 25 cc. of the preparation, measured at 25°, in a distilling flask of about 200-cc. capacity, provided with a high side tube connected with a suitable condenser; add 50 cc. of distilled water, distill into a 50-cc. graduated flask, at such a rate that about 48 cc. of distillate will be received in $\frac{1}{2}$ hr.; if volatile products other than alcohol are known to be absent, bring to the original temperature and dilute to 50 cc. with water of the same temperature.

In very accurate work, it is best that the volume of the sample and of the distillate be measured at the same temperature, *i.e.*, 15.56°. When the temperatures differ, the volume of the sample used and of the distillate collected are not the same, since the coefficient of expansion of alcohol is much greater than that of water. In the volume percentages for alcohol, it is assumed that the volumes are measured at 15.56°.

2. Determine the specific gravity of the distillate at 25° by means of a pycnometer, and find the per cent of absolute alcohol by volume by reference to the alcoholometric table in the U.S.P., page 1011, and multiply by the dilution factor to find the per cent of alcohol in the original preparation.

The specific gravity of the distillate may be determined by means of either the Sprengel-Ostwald or the Geissler pycnometer, as previously described. The latter instrument is the more satisfactory, since the temperature of the alcoholic solution can be determined while the specific-gravity determination is being made.

The method of recording the data and calculating the results is illustrated in the example:

Volume of sample at 25° = 25 cc.
Volume of distillate at 25° = 50 cc.
Dilution factor = $^{50}/_{25}$ = 2

The specific gravity of the distillate at 25°/25° found = 0.9639.

By referring to the alcoholometric table, the percentage by volume of alcohol in the distillate is found to be 28 + 0.7 = 28.7. This percentage is based on the apparent specific gravity of alcohol at 15.56°/15.56°. The per cent of absolute alcohol by volume contained in the sample tested is found by multiplying the per cent found in the distillate by the dilution factor, or 28.7 × 2 = 57.4 per cent. The corresponding per cent of absolute alcohol by weight may be found by consulting the table on page 1011 of the U.S.P.; *e.g.*, 57 and 58 per cent of alcohol by volume are found to correspond to 49.19 and 50.17 per cent of alcohol by weight, respectively. Therefore, 57.4 per cent of alcohol by volume corresponds to 49.58 per cent by weight.

The amount of alcohol present in various preparations such as fluidextracts and tinctures is subject to considerable variation due to the varying proportions of moisture and extractive contained in the drug used and to the methods of extraction and concentration employed in their preparation. The official standards, therefore, generally give a range of alcohol content for preparations of this type; *e.g.*, fluidextract of belladonna leaf is required to contain 57 to 63 per cent of alcohol by volume.

2. By the Use of the Westphal Balance. The specific gravity of liquids may be determined quickly and with a fair degree of accuracy by means of the apparatus known as the *Westphal balance.* The mechanism of this device is based upon the generally known law of physics that a body immersed in a liquid is buoyed up by a force equal to the weight of the liquid displaced. This apparatus (Figs. 32, 33, and 34) consists of a beam balanced on a knife-edge *A* with a sinker or plummet *B*, suspended from one end and counterpoised by a fixed brass weight *C* at the other. The distance between the knife-edge *A* and the plummet support *D* is graduated into ten divisions of equal length. The plummet, which is made of glass and provided with a thermometer, is made of such size that it will displace exactly 5 Gm. of water at a given temperature, usually 15.56°. The plummet and the platinum wire by which it is suspended are usually made of a definite weight, so that they are interchangeable on different balances.

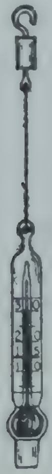

FIG. 32. Plummet.

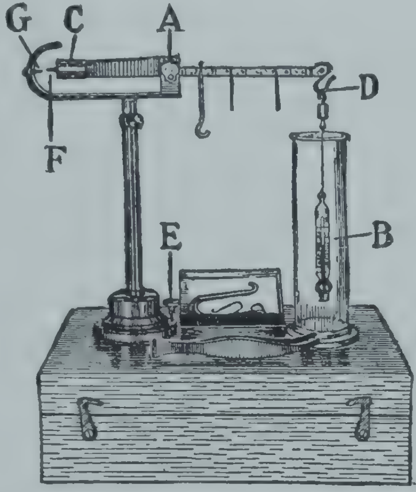

FIG. 33. Westphal specific gravity balance:

FIG. 34. Riders.

Exercise 111

Object. To Determine the Specific Gravity of a Volatile Oil.

Materials Required. A Westphal balance.
A 50-cc. cylindrical graduate.
50 cc. of volatile oil.

Procedure. 1. Balance the apparatus in air.

To balance the apparatus, place it upon a firm, level table and be sure that the leveling screw in the base is directly beneath the arm that supports the plummet, compare the height of the beam with that of the cylinder which is to contain the oil, lengthen or shorten the standard, if necessary, and adjust the level of the beam by means of the leveling screw *E* until the points *F* and *G* exactly coincide.

2. Suspend the plummet in water, contained in the glass cylinder, place the largest rider weight on the hook to which the plummet is attached, and balance the instrument again by turning the leveling screw.

When determinations are made at the exact temperature fo which the instrument is designed, no adjustment should be neces sary at this point, but in taking the specific gravity at 25°, a sligh adjustment is required to correct for the expansion of the liquid a the higher temperature.

3. Dry and clean the plummet and cylinder, fill the cylinder with the oil t the same level as that reached by the water in balancing the apparatus, lift th plummet, and place the cylinder beneath the end of the beam. Replace the plummet so that it hangs free in the oil without touching the cylinder wall an place the riders on the beam until balance is restored.

The temperature of the oil (25°) at which the final adjustment of the balance is made should be the same as that of the water used to standardize the apparatus. The specific gravity is read directl from the position of the riders on the beam; *e.g.*, if the largest rider is at 9, the second at 7, the third at 5, and the fourth at 2, the specific gravity of the oil is 0.9752. Readings should always be made to four decimal places; *i.e.*, all four sizes of riders should be used.

3. By Weighing a Solid in Air, in Water, and in the Liquid. Plummets or sinkers of various forms made of sealed glass tubes partly filled with mercury or of some metal such as platinum have been devised for specific-gravity determinations of this type, but a brass weight will suffice for the determination of the specific gravity of liquids that do not attack the brass. For example, if a piece of brass weighs 10 Gm. in air, 8.824 Gm. in water, and 9 Gm. in a liquid of unknown specific gravity, the specific gravity of the last can be calculated from a simple proportion as follows:

$$1.176:1::1':X \qquad X = 0.85$$

where 1.176 is the loss of weight in water ($10 - 8.824 = 1.176$) or the weight of the water displaced; 1 is the loss of weight in the liquid; 1′, the specific gravity of water; and X, the specific gravity of the unknown liquid.

The principles involved and the method of making the determination are similar to those given in detail under the determination of the specific gravity of solids heavier than, and insoluble in, water (page 410).

1. **By the Use of Hydrometers.** Hydrometers are instruments used to determine quickly the approximate specific gravity or density of liquids by flotation. They usually consist of a sealed glass tube weighted with mercury or shot and frequently are provided with a thermometer, so that the temperature at which the specific gravity is determined may be read at the same time. They are variously called *alcoholometers*, *lactometers*, *urinometers*, etc., according to the use for which they are designed. They are either constructed to read specific gravity directly, as, for example, the hydrometers for general use and lactometers, or they are graduated according to some arbitrary scale of degrees, as in the case of the Baumé and Twaddell hydrometers. Specific-gravity hydrometers are usually made in sets of four or six, each instrument being calibrated to cover a different range in specific gravities, so that the complete set will suffice to determine the specific gravity of almost any liquid: *e.g.*, the different instruments in the set of four are calibrated to read from 0.600 to 1.000, from 1.000 to 1.400, from 1.400 to 1.800, and from 1.800 to 2.200.

When making a specific-gravity determination with a hydrometer, the thoroughly cleaned instrument should be allowed to sink in the liquid gradually until it is at rest, then depressed about 1 in. to moisten a portion of the stem, allowed to come to equilibrium, and the reading taken.

Exercise 112

Object. To Prepare 200 cc. of Sulfuric Acid Containing 10 Gm. of H_2SO_4 per 100 cc.

Materials Required. 20 cc. of concentrated H_2SO_4.

A hydrometer calibrated from specific gravity 1.000 to 1.400 at 25°.

Procedure. Dilute 20 cc. of pure concentrated sulfuric acid to 100 cc. with distilled water, allow the resulting solution to acquire a temperature of 25°, and take its specific gravity as accurately as possible with the hydrometer.

If the specific gravity of the solution is found to be 1.2603, it contains 35 per cent of H_2SO_4 as found by referring to the table, page 801 of the U.S.P. XII. The weight of the prepared acid solution to be diluted will then be $\frac{10 \times 2 \times 100}{35} = 57.1430$ Gm. This weight of solution containing 35 per cent of H_2SO_4 when diluted to 200 cc. will form a solution containing 10 Gm. of H_2SO_4 per 100 cc.

Solutions prepared as directed above are subject to slight inaccuracies of concentration, since specific-gravity determinations by means of hydrometers are not exact, but if the specific gravity is determined accurately by means of a pycnometer, the concentration of a solution prepared in this manner will be sufficiently accurate for all ordinary qualitative purposes.

METHODS USED TO DETERMINE THE SPECIFIC GRAVITY OF SOLIDS

1. Solids Heavier Than and Insoluble in Water. *A.* Weigh the substance in air and then in water.

According to the law of Archimedes, a body suspended in a fluid is buoyed up by a force equal to the weight of the fluid displaced. The weight of a solid in air, therefore, less its weight in water, gives the weight of an equal volume of water, since the volume of water displaced is equal to the volume of the solid. If the weight in air is divided by the loss of weight in water, the result is the specific gravity of the substance at the temperature at which the determination is made. For example: Suppose that a piece of brass weighs 10.0040 Gm. in air at 25° and 8.8010 Gm. immersed in water at 25°. The weight in air divided by the loss of weight, 1.2030 Gm., gives the specific gravity of the brass at 25°/25° or $10.0040/1.2030 = 8.32$.

B. If the substance is in small pieces or is a powder, the specific gravity may be determined as illustrated in the following exercise.

Exercise 113

Object. To Determine the Specific Gravity of Powdered Barium Sulfate.

Materials Required. 7 to 10 Gm. of powdered barium sulfate.
A small volumetric flask (25 or 50 cc.).

Procedure. 1. Weigh about 10 Gm. of the powdered barium sulfate accurately.

2. Accurately weigh the small volumetric flask filled exactly to the graduation mark with recently boiled distilled water at 25°.

3. Introduce the powdered barium sulfate into the flask quantitatively,

remove any air occluded in the powder by agitating the mixture, draw off the water with a pipette until the level of the liquid is again exactly at the graduation mark when the liquid is at 25°, and weigh the flask and its contents accurately.

It is evident that a volume of water equal to that of the barium sulfate has been displaced and removed from the flask. The specific gravity of the barium sulfate may then be calculated, *e.g.*:

Weight of powdered barium sulfate = 8.0 Gm.
Weight of flask filled with water = 80 Gm.
Weight of flask, barium sulfate, and water = 85 Gm.

80 + 8 − 85 = 3 Gm., the weight of the volume of water displaced by the barium sulfate; and $^8/_3$ = 2.6666, the specific gravity of the barium sulfate.

2. Solids Lighter Than and Insoluble in Water. *A. By Weighing in Water.* If a substance is lighter than water, it will float, and its volume cannot be obtained by weighing it in water unless it is completely submerged by means of a weight or sinker heavier than water. The method of determining and calculating the specific gravity of a substance of this type is illustrated in the following exercise.

Exercise 114

Object. To Determine the Specific Gravity of Camphor.

Materials Required. A piece of camphor (2 to 4 Gm.).

A suitable sinker such as a 2-Gm. weight.

Procedure. 1. Weigh the piece of camphor in air.

2. Weigh the sinker immersed in water.

3. Attach the sinker to the piece of camphor and find their combined weight when immersed in water.

Since the camphor is lighter than water, the weight of the sinker and camphor is less than the weight of the sinker alone. The difference between the weight of the sinker and camphor in water and the weight of the sinker alone in water plus the weight of the camphor in air is a measure of the water displaced by the camphor and also a measure of its buoyant power, *e.g.*:

Weight of the camphor in air = 4 Gm.
Weight of the sinker in water = 1.6 Gm.
Weight of the camphor and sinker in water = 1.5 Gm.

The difference between the weight of the sinker in water and the weight of the sinker and camphor in water = 1.6 − 1.5 = 0.1 Gm.

The weight of the camphor in air plus this difference of 0.1 Gm. is the weight of a volume of water equal to the weight of the piece of camphor or 4 + 0.1 = 4.1 Gm. of water displaced.

Since the specific gravity of a substance is found by dividing its weight by the weight of an equal volume of water, the specific gravity of the camphor is 4/4.1 = 0.9756.

B. By the Flotation Method. This method consists of bringing the substance into equilibrium with a liquid of known specific gravity. It is used in the determination of the specific gravity of such substances as white and yellow wax and spermaceti, and by food and drug analysts to determine the specific gravities of certain fats and oils.

Exercise 115

Object. To Determine the Specific Gravity of Yellow Wax.

Materials Required. About 0.25 Gm. of yellow wax.
25 cc. of alcohol.
A pycnometer.

Procedure. 1. Melt the wax at a low temperature and allow it to fall into separated drops from just above the surface into alcohol that has been warmed to from 45 to 50°. Allow the globules to remain in the alcohol until it has cooled spontaneously to room temperature (20 to 25°); then remove the wax and keep it at room temperature for 24 hr.

The melted wax separates in globules free from occluded air, which solidify when the mixture is cooled. The wax, which has a high coefficient of expansion, is kept at room temperature for 24 hr. to permit the globules to acquire their normal volume.

2. Prepare a mixture of four volumes of alcohol and enough distilled water to make ten volumes, and allow it to stand until free from air bubbles. Moisten the globules of wax with distilled water by means of a brush, and place them by means of forceps in the alcohol solution just prepared and contained in a beaker. Then add alcohol or air-free distilled water, as required, to the mixture kept at 25°, until the globules of the wax float and rest indifferently in the liquid. Finally, determine the specific gravity of the alcohol-water mixture. The figure thus obtained represents the specific gravity of the wax examined.

The wax globules are moistened with water, so that the water-alcohol mixture will come in contact with all portions of the globules without occluding air at the interface. When the globules are in equilibrium in the alcoholic solution, their weight must be equal to the weight of liquid that they displace or they would rise to the top of the liquid and float, or sink to the bottom of the beaker. Consequently, when such equilibrium is attained, the specific gravity of the wax and that of the liquid are equal, and a determination of the specific gravity of the liquid is a measure of the specific gravity of the wax.

3. Solids Heavier Than and Soluble in Water. The specific gravity of solids heavier than and soluble in water may be determined by weighing the solid in air and then in some liquid of known specific gravity in which the solid substance is insoluble, such as oil of turpentine, chloroform, benzene, etc. Thus, the weight of a piece of alum in air divided by its loss in weight when weighed immersed in oil of turpentine gives the density of the alum relative to the oil of turpentine, and if the specific gravity of the oil of turpentine is known, that of the alum can be calculated, *e.g.*:

Weight of the alum in air = 10 Gm.
Weight of the alum in oil of turpentine = 6.5 Gm.
Specific gravity of the oil of turpentine = 0.860

$10 - 6.5 = 3.5$ Gm., loss in weight of the alum in the oil of turpentine equal to the weight of an equal volume of the latter.

$$3.5:X::0.86:1 \qquad X = 4.070 \text{ Gm.}$$

where X is the weight of a volume of water equal to the volume of the oil of turpentine displaced, and $10/4.070 = 2.4570$, the specific gravity of the alum.

4. Solids Lighter Than and Soluble in Water. The specific gravity of substances lighter than and soluble in water may be determined by weighing the substance with a sinker in some liquid of known specific gravity in which the substance is not soluble. For example, the specific gravity of lithium may be determined from the data below as follows:

Weight of the lithium in air = 2.0 Gm.
Weight of the sinker in air = 5.0 Gm.
Specific gravity of the sinker (brass) = 8.4
Specific gravity of liquid (kerosene) = 0.830
Weight of lithium and sinker in kerosene = 3.8 Gm.

The density X of the sinker referred to kerosene as a standard is

$$0.830:1::8.4:X \qquad X = 10.1205$$

The weight Y of the sinker in kerosene may be calculated,

$$\frac{5.0}{5.0 - Y} = 10.1205 \qquad Y = 4.5095$$

and the density of lithium compared to that of kerosene is

$$\frac{2}{2 + 4.5060 - 3.8} = 0.7391$$

The specific gravity Z of the lithium is, therefore,

$$0.830:1::Z:0.7391$$
$Z = 0.6135$, the specific gravity of the lithium.

Questions and Problems

1. Define the terms *specific gravity*, *apparent specific gravity*, and *true specific gravity*.

2. By what different methods might the specific gravity of alcohol be determined?

3. A 10-Gm. weight of brass, specific gravity 8.4, submerged in glycerin weighed 8.5135 Gm. What is the specific gravity of the glycerin?

4. Given the specific gravity of sulfuric acid at 15° as 1.8422 compared with water at 15°, what is its specific gravity referred to water at 21°? (See U.S.P. XII.)

5. How much nitric acid, specific gravity 1.45, and how much water must be mixed to form 1 liter of nitric acid, specific gravity 1.20?

6. Calculate the specific gravity of a piece of sandalwood from the following data:

Weight in air = 10.100 Gm.
Weight of sinker in water = 12.200 Gm.
Weight of sinker and sandalwood in water = 11.900 Gm.

TABLE 62. OFFICIAL SUBSTANCES WITH SPECIFIC GRAVITIES

Substance	Specific gravity, 25°
U.S.P.	
Alcohol	(n.m.t.) 0.816*
Alcohol, diluted	0.935 to 0.937*
Almond oil, expressed	0.910 to 0.915
Amphetamine	0.930 to 0.936
Amyl nitrite	0.865 to 0.875
Amylene hydrate	0.803 to 0.807
Anise oil	0.978 to 0.988
Aspidium oleoresin	(n.l.t.) 1.00
Benzyl benzoate	1.116 to 1.120
Butopyronoxyl	1.052 to 1.060
Castor oil	0.945 to 0.965
Cherry juice	1.045 to 1.075
Chloroazodin solution	1.154 to 1.158
Chloroform	1.474 to 1.478
Cinnamon oil	1.045 to 1.063
Clove oil	1.038 to 1.060
Cod liver oil	0.918 to 0.927
Collodion	0.765 to 0.775
Coriander oil	0.863 to 0.875
Corn oil	0.914 to 0.921
Cottonseed oil	0.915 to 0.921
Cresol	1.030 to 1.038
Dimercaprol	1.238 to 1.240
Dimethyl phthalate	1.188 to 1.192
Ether	0.713 to 0.716
Ethohexadiol	0.936 to 0.940
Ethyl oxide	0.713 to 0.716
Eucalyptol	0.921 to 0.924
Eugenol	1.064 to 1.070
Fennel oil	0.953 to 0.973
Glycerin	(n.l.t.) 1.249
Glyceryl triacetate	1.154 to 1.158
Halibut liver oil	0.920 to 0.930
Lactic acid	About 1.206
Lavender oil	0.875 to 0.888
Lemon oil	0.849 to 0.855
Methyl salicylate (natural)	1.176 to 1.182
Methyl salicylate (synthetic)	1.180 to 1.185
Myristica oil (East Indian)	0.880 to 0.910
Myristica oil (West Indian)	0.854 to 0.880
Nikethamide	1.058 to 1.066
Olive oil	0.910 to 0.915
Orange oil	0.842 to 0.846
Peanut oil	0.912 to 0.920
Peppermint oil	0.896 to 0.908
Persic oil	0.910 to 0.923
Peruvian balsam	1.150 to 1.170
Petrolatum	0.815 to 0.880†
Petrolatum, liquid	0.860 to 0.905
Petroleum benzin	0.634 to 0.660
Polyethylene glycol 400	1.110 to 1.140
Polysorbate 80	1.06 to 1.10
Propylene glycol	1.035 to 1.037
Raspberry juice	1.025 to 1.045
Rice polishings extract	1.28 to 1.32‡
Rose oil	0.848 to 0.863§
Rosemary oil	0.894 to 0.912
Sassafras oil	1.065 to 1.077
Sesame oil	0.916 to 0.921
Spearmint oil	0.917 to 0.934
Tetrachlorethylene	1.603 to 1.615
Theobroma oil	0.858 to 0.864¶
Trichloroethylene	1.456 to 1.462
Triethanolamine	1.1204 to 1.1284
Vinyl ether	0.767 to 0.771
N.F.	
Acetic acid, diluted	About 1.008
Acetone	(n.m.t.) 0.789
Alcohol, dehydrated	0.798*
Alcohol rubbing compound	0.8797 to 0.8874* 0.8691 to 0.8771[ba]
Almond oil, bitter	1.038 to 1.060
Aluminum chloride solution	About 1.2
Anethole	0.983 to 0.988
Antiseptic solution	About 0.971
Aromatic solution, alkaline	About 1.04
Benzaldehyde	1.041 to 1.046
Benzyl alcohol	1.040 to 1.050
Bergamot oil	0.875 to 0.880
Birch tar oil, rectified	0.886 to 0.950
Brandy	0.921 to 0.933
Camphor spirit	0.824 to 0.826
Caramel	(n.l.t.) 1.30
Caraway oil	0.900 to 0.910
Carbon tetrachloride	1.588 to 1.590

Table 62. Official Substances with Specific Gravities. *(Continued*

Substance	Specific gravity, 25°	Substance	Specific gravity, 25°
N.F.		N.F.	
Cardamom oil	0.917 to 0.947	Merbromin solution, surgical	0.9110 to 0.9140
Castor oil capsules	0.945 to 0.965[c]	Myrcia oil	0.950 to 0.990
Chaulmoogra oil	0.940 to 0.960	Nitric acid	1.41
Chenopodium oil	0.950 to 0.980	Nitromersol solution	1.005 to 1.010
Cinnamaldehyde	1.048 to 1.052	Nitromersol tincture	0.9050 to 0.9150
Coconut oil	0.918 to 0.923	Orange, bitter, oil	0.845 to 0.851
Copaiba	0.930 to 0.995	Orange flower oil	0.863 to 0.880
Creosote	(n.l.t.) 1.076	Petrolatum, light, liquid	0.828 to 0.880
Creosote carbonate	(n.l.t.) 1.145	Phosphoric acid	1.71
Ether spirit	0.784 to 0.794	Phosphoric acid, diluted	1.057
Ethereal oil	About 0.9	Pimenta oil	1.018 to 1.048
Ethyl acetate	0.894 to 0.898	Pine needle oil (dwarf)	0.853 to 0.871
Ethyl nitrate spirit	(n.m.t.) 0.823	Pine oil	0.927 to 0.940
Eucalyptus oil	0.905 to 0.925	Potassium iodide solution	1.700
Ferric chloride solution	1.29 to 1.35	Resorcinol monoacetate	1.203 to 1.207
Ferric chloride tincture	1.00	Rosin	1.07 to 1.09
Ferric subsulfate solution	1.548	Santal oil	0.965 to 0.980
Ferrous iodide syrup	1.37	Sherry wine	0.985 to 1.030*
Formic acid	About 1.06	Silver nitrate solution, ammoniacal	1.48
Glyceryl trinitrate spirit	0.814 to 0.820	Sodium hypochlorite solution, diluted	1.025
Guaiacol (liquid)	1.112	Sodium phosphate solution	1.385
Guaiacol (melted solid)	1.132	Sulfuric acid	1.84
Hamamelis water	0.979 to 0.982	Sulfuric acid, diluted	1.067
Honey	(n.l.t.) 1.099[d]	Tar, Juniper	0.950 to 1.055
Iron, peptonized, and manganese solution	1.029	Tar oil, rectified	0.960 to 0.990
Isopropyl alcohol	0.7828 to 0.7869	Thyme oil	0.910 to 0.935
Isopropyl alcohol, rubbing compound	0.8690 to 0.8790	Turpentine oil	0.854 to 0.868
Juniper oil	0.854 to 0.879	Turpentine oil, rectified	0.853 to 0.862
Lead subacetate solution	1.25	Undecylenic acid	0.910 to 0.913
Linseed oil	0.925 to 0.935	Whisky	0.923 to 0.935
Malt extract	1.350 to 1.430		
Merbromin solution	1.010 to 1.015		

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* 15.56°.

† 60°.

‡ 27.5°.

§ 30°.

¶ 100°.

[a] Made with specially denatured alcohol, Formula 23-G.

[b] Made with specially denatured alcohol, Formula 23-H.

[c] Specific gravity of the oil.

[d] Diluted with 2 volumes of water.

(n.l.t.) = not less than. (n.m.t.) = not more than.

7. How many grams of water will a pycnometer calibrated to hold 100 Gm. of water at 25°/25° and 760 mm. pressure contain at 19° and 740 mm. pressure? (See U.S.P. XII.)

8. Give the specific gravity of alcohol compared with water at 15.56° and 760 mm. pressure in a solution containing 16.5 per cent of alcohol by volume. (See U.S.P.)

9. Briefly state how the specific gravity of each of the following might be determined with comparative accuracy: olive oil, benzene, powdered borax, granular zinc, and cacao butter.

The approximate specific gravities for many official substances, such as acetone, acids, and numerous preparations, are given in the U.S. Pharmacopoeia and the National Formulary simply as information for the conversion of volume to weight. When the minimum or maximum specific gravity or a range of specific gravity is given, it is a definite constant, of value in determining the identity, purity, and concentration of the substance. Those substances for which definite specific gravity limits are given in the official standards are shown in Table 62.

Chapter 21

MELTING, CONGEALING, AND BOILING TEMPERATURES

Melting Point. When an amorphous substance is heated, it gradually softens, eventually acquiring the properties characteristic of a liquid, but no definite point of transition from the solid to the liquid state occurs during the process of heating. When a crystalline solid is heated, there is a sharp change from the solid to the liquid state at a definite temperature, this temperature being known as the *melting point* of the substance.

The melting point of a compound may be defined as *the temperature at which the liquid and solid phases of the compound are in equilibrium.* Each pure fusible compound has a fixed and definite melting point, and this constant is often used to determine the purity of a solid, since the introduction of even a small amount of impurity lowers the melting point to a considerable extent. If appreciable quantities of impurities are present, the melting point is not sharp but ranges over a number of degrees and is usually low. In the official standards, the melting range or temperature of a substance is defined as *those points of temperature within which or at which the solid coalesces and is completely melted, when determined as directed.* For the determination of the melting range, the official substances are divided into four classes, *viz.*, (1) substances that can be readily reduced to a powder; (2) substances, such as fats, fatty acids, and waxes, that cannot be reduced to a powder readily; (3) petrolatum; (4) paraffin.

The apparatus (Fig. 35) required to determine the melting range of substances in Class 1 or 2 consists of the following parts: (1) a thin-walled glass tube about 3 or 4 cm. in diameter and about 12 cm. long; (2) a stirring rod bent into a circle at one end to fit the above tube and at an angle at the other end to permit easy manipulation;

3) a thermometer covering the desired range of temperature and tandardized for 76 mm. or 3 in. immersion to compensate for the mergent mercury column; (4) an auxiliary thermometer to make he emergent stem correction. This thermometer is no longer equired in the official methods since the official melting range thermometer is standardized to compensate for the error introduced by mergent mercury column; (5) a capillary glass tube about 9 cm. ong and from 0.8 to 1.2 mm. internal diameter, the walls from 0.2 to 0.3 mm. thick and the tube closed at one nd.

Any other apparatus or method, capable of equal accuracy, may be used. Six U.S.P. Melting Point Reference Standards are available and may be used for checking the accuracy of a method or apparatus.

For temperatures up to 200°, a purified, concentrated sulfuric acid is a suitable bath. For higher temperatures, up to about 350°, a pure grade of cottonseed oil (almost colorless) will serve for a limited number of determinations. Other, though less desirable, substitutes for sulfuric acid for use at high temperatures are: (1) a pure grade of paraffin which has been freshly distilled; (2) clean, white, hydrogenated cottonseed oil. A very satisfactory bath is prepared by cautiously boiling together, for from 5 to 10 min. under a hood, a mixture of 70 parts of sulfuric acid and 30 parts of potassium sulfate, stirring constantly until the potassium sulfate is completely dissolved.

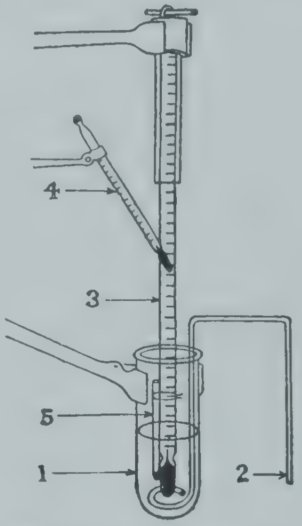

FIG. 35. Apparatus for taking melting points.

The following exercises illustrate the methods used to determine the melting ranges of official substances.

Exercise 116

Object. To Determine the Melting Range of Salicylic Acid.

Materials Required. A melting-point apparatus, as described above.
20 to 30 cc. of concentrated sulfuric acid.
About 0.05 Gm. of salicylic acid.

Procedure. 1. Reduce the salicylic acid to a fine powder and dry it at 100° for 2 hr.

The material is dried because some substances that contain water of crystallization or moisture must be rendered anhydrous to prevent liquefaction when heated through the solvent action of water, and because water as an impurity lowers the melting point.

2. Place sufficient sulfuric acid in the test tube to fill it to a depth of about 5 cm., so that the thermometer bulb may be immersed about 3 cm. below the upper surface of the bath and leave about 2 cm. of the liquid below the lower end of the bulb.

This arrangement prevents unequal heating of the different parts of the thermometer bulb.

3. Introduce enough of the finely powdered, dry salicylic acid into the capillary tube to form a column about 0.3 cm. in length.

The salicylic acid may be packed into the sealed end of the tube by tapping the open end of it into a little mound of the powder, then inverting the tube and tapping it gently on a solid surface, or by drawing the broad side of a triangular file across the surface of the tube just below the powder.

4. Attach the charged capillary tube to the thermometer by wetting both with the acid of the bath, and the tube will then be held in position by capillary attraction, or attach the capillary tube by means of a piece of platinum wire, in such a position that the column of salicylic acid is centrally located by the side of the thermometer bulb. If a thermometer standardized for stem immersion is used the auxiliary thermometer is not required; otherwise attach the auxiliary thermometer so that the center of its bulb is as close as possible to the stem of the main thermometer at a point midway between the surface of the bath and the 160° graduation mark. Heat the acid bath by means of a free Bunsen flame until a temperature of about 130° is reached, then carefully regulate the rise in temperature to about 3° per minute until a temperature 3° below the expected melting point is attained, and then carefully regulate the rise in temperature to about 1° per minute until melting is complete. Stir the bath constantly throughout the heating. Record the melting interval temperature and the temperature registered on the auxiliary thermometer if it is used.

The temperature at which the column of salicylic acid is observed to collapse definitely in the capillary tube is regarded as the beginning of the melting; the temperature at which the salicylic acid becomes liquid throughout is regarded as the end of the melting, and the interval between the temperatures at which the melting begins and ends is taken as the melting range of salicylic acid.

The rate of rise in the temperature of the bath can be regulated easily if the burner is held in the hand so that more or less heat can be applied as required. Since heat from the burner may affect the temperature registered on the auxiliary thermometer, it is advisable to construct a movable platform of stiff asbestos paper around the main thermometer and adjust it to a position just below the bulb of the auxiliary thermometer.

If a standardized thermometer is used the reading of the melting point thermometer need not be corrected. Otherwise the temperature observed on the auxiliary thermometer is used to correct for the contraction of the thread of mercury above the bath by the use of the following formula:

Correction $= 0.00015 \times N(T - t)$, where N represents the number of degrees from the surface of the bath to the melting point, T the temperature at the end of melting, and t the temperature registered on the auxiliary thermometer. The correction is added to the observed reading of the main thermometer; *i.e.*, if in the determination of the melting point of salicylic acid the main thermometer is immersed in the bath to the 30° graduation mark, the acid is completely melted at 156.5°, and the temperature registered on the auxiliary thermometer is 40°, the correction for the melting point temperature would be

$$0.00015 \times 126.5(156.5 - 40) = 2.59^\circ$$

and the corrected melting point becomes $156.5 + 2.59 = 159.1^\circ$.

The melting range of a substance that cannot be readily reduced to a powder is determined in the same manner with the following exceptions: The substance is melted at as low a temperature as possible and drawn into a capillary tube, open at both ends, to a depth of about 1 cm. The charged tube is allowed to cool at 10° or at a lower temperature for 24 hr. or in contact with ice for 2 hr. The melting range of the substance is then determined by attaching the charged tube to a standard thermometer as described in the above exercise, or by means of a piece of platinum wire and heating in a suitable bath so that the rate of rise of temperature does not exceed 0.5° per minute for the last 5° before the expected melting range is reached. The temperature at which the substance is observed to rise in the capillary tube is taken as the melting range. Melting-range determinations by the above method are generally performed on substances that are mixtures and that do not give the sharp melting-range characteristic of pure organic compounds.

Questions and Problems

1. Look up the melting ranges given for each of the following substances in the U.S.P., and explain briefly why an interval of melting-range temperatures is permitted in some cases while fixed melting points are given in others: (*a*) paraffin; (*b*) petrolatum; (*c*) benzoic acid; (*d*) menthol; (*e*) cocaine; (f) progesterone.

2. Why should thermometers for use in melting-range determinations be made of annealed glass? (See U.S.P.)

3. How might the emergent stem correction for the main thermometer be determined by interpolation from a correction curve when a number of melting ranges are to be determined? (See U.S.P.)

4. What per cent of error would be introduced in the determination of the melting range of salicylic acid as found, if uncorrected?

5. Given two samples that show the same melting range, how might you determine whether or not they were the same substance?

Congealing Temperature. The congealing or solidifying temperature is a physical constant, characteristic of certain liquids, such as anise oil, eucalyptus oil, and the fatty acids of fixed oils, that may serve as a criterion of purity and quality just as the melting range of a solid does, since the presence of impurities also causes a depression of the congealing temperature. The method for the determination of congealing temperatures is as follows:

Unless otherwise directed, about 10 cc. of the liquid to be tested is placed in a dry test tube of from 18 to 20 mm. internal diameter, which is then cooled in water or a suitable freezing mixture, the temperature of which should be about 5° lower than the supposed congealing temperature of the liquid. To induce crystallization, rub the walls of the tube with a standard thermometer, or add a small crystal of the substance being tested. By alternate immersion of the tube in the bath, or removal from the bath, and constant

TABLE 63. MELTING RANGES OF OFFICIAL SUBSTANCES

Substance	Melting range, °C.
U.S.P.	
Acetanilid	114 to 116
Acetophenetidin	134 to 136
Aminopyrine	107 to 109
Atropine	114 to 116
Atropine sulfate	(n.l.t.) 188*
Barbital	188 to 192
Benzoic acid	121 to 123
Bishydroxycoumarin	287 to 293
Butacaine sulfate	100 to 103
Caffeine	235 to 237.5*
Calciferol	115 to 118
Camphor	174 to 179
Carbachol	202 to 205
Chloramphenicol	149 to 153
Chlorobutanol	(n.l.t.) 76
Chloroguanide hydrochloride	248 to 252
Chlorophenothane	103 to 109
Chloroquine phosphate	193 to 195 or 215 to 218
Cholesterol	147 to 150
Cocaine	96 to 98
7-Dehydrocholesterol, activated	82 to 86
Desoxycorticosterone acetate	154 to 160
Diethylstilbestrol	169 to 172
Diphenhydramine hydrochloride	166 to 170
Ephedrine hydrochloride	217 to 220
Estradiol	173 to 179
Estradiol benzoate	190 to 195
Estrone	255 to 262
Ethisterone	266 to 273
Ethyl aminobenzoate	88 to 90
Eucatropine hydrochloride	183 to 190
Hexylresorcinol	62 to 67
Histamine phosphate	126 to 132
Homatropine methylbromide	190 to 198
Iodoalphionic acid	160 to 164†
Menadione	105 to 107
Menthol (l-)	41 to 43
Meperidine hydrochloride	186 to 189
Methacholine chloride	170 to 173*
U.S.P.	
Methamphetamine hydrochloride	171 to 175
Methylparaben	125 to 128
Methyltestosterone	161 to 166
Naphazoline hydrochloride	255 to 260
Neocinchophen	74 to 77
Neostigmine methylsulfate	142 to 145
Nicotinamide	128 to 131
Nicotinic acid	234 to 237
Para-aminobenzoic acid	186 to 189
Paraffin	47 to 65
Pentaquin phosphate	188 to 192
Pentylenetetrazol	57 to 59
Petrolatum	38 to 60
Phenobarbital	174 to 178
Phenolphthalein	(n.l.t.) 258
Phenylephrine hydrochloride	139 to 142
Phthalylsulfathiazole	272 to 277†‡
Picrotoxin	198 to 200
Pilocarpine hydrochloride	200 to 203
Pilocarpine nitrate	170 to 173
Piperocaine hydrochloride	172 to 175
Polyethylene glycol 400 monostearate	30 to 34
Polyethylene glycol 4000	53 to 56
Procaine hydrochloride	153 to 156
Progesterone (α-form)	127 to 131
Progesterone (β-form)	121§
Propylparaben	95 to 98
Propylthiouracil	218 to 221
Resorcinol	109 to 111
Saccharin	226 to 230
Salicylic acid	158 to 161
Scopolamine hydrobromide	195 to 199*‡
Spermaceti	42 to 50
Stearyl alcohol	56 to 60
Sulfadiazine	252 to 256
Sulfamerazine	234 to 238
Sulfanilamide	164.5 to 166.5
Sulfaguanidine	190 to 193*
Sulfathiazole	200 to 204

TABLE 63. MELTING RANGES OF OFFICIAL SUBSTANCES. *(Continued)*

Substance	Melting range, °C.
U.S.P.	
Testosterone	152 to 156
Testosterone pellets	152 to 156
Testosterone propionate	118 to 122
Tetracaine hydrochloride	147 to 150
Theophylline	270 to 274
Tribromoethanol	79 to 82
Trimethadione	45 to 47
Tripelennamine hydrochloride	188 to 192
Urea	131 to 133
Urethane	48 to 50
Vanillin	81 to 83
Wax, yellow and white	62 to 65
Wool fat	36 to 42
N.F.	
Amobarbital	156 to 158.5
Anthralin	175 to 181
Antipyrine	110 to 112.5
Arecoline hydrobromide	170 to 175
β-naphthol	120 to 123
Butyl aminobenzoate	57 to 59
Calcium levulinate	119 to 125‡
Camphor, monobromated	74 to 76
Carbromal	116 to 119
Cetyl alcohol	45 to 50
Chlorothymol	59 to 61
Cincophen	213 to 216
Coconut oil	22 to 25

Substance	Melting range, °C.
N.F.	
Codeine	154 to 156*
Coumarin	68 to 70
Dehydrocholic acid (oral use)	232 to 242
Dehydrocholic acid (parenteral use)	237 to 242
Dibucaine hydrochloride	95 to 100
Glyceryl monostearate	56 to 58
Guaiac	85 to 90
Guaiacol (solid)	28§
Iodoform	115‡§
Lard	36 to 42
Mandelic acid	118 to 120
Oleyl alcohol	13 to 19
Phenacaine hydrochloride	(n.l.t.) 190*
Phenylmercuric chloride	250 to 252
Phenylmercuric nitrate	175 to 185
Phenyl salicylate	41 to 43
Pyrogallol	130 to 133
Salicin	199 to 202
Salicylanilide	136 to 138
Santonin	170 to 173
Succinchlorimide	148 to 149
Suet, prepared	45 to 50
Sulfapyridine	191 to 193
Terpin hydrate	102 to 105*
Thymol	48 to 51
Trinitrophenol	121 to 123

* After drying.
† Decomposition.
‡ Preheated bath.
§ About.
(n.l.t.) = not less than.

stirring with the thermometer, the temperature is so adjusted that the greater part of the liquid gradually congeals. *The highest temperature remaining constant for about* 1 *min. during the congelation is defined as the congealing temperature.*

The solidification temperature of fatty acids is commonly referred to as the *titer*. The official procedure for the determination of the solidification of fatty acids is illustrated in the following exercise.

Exercise 117

Object. Determination of the Solidification Temperature of the Fatty Acids of Cottonseed Oil.

Materials Required. 50 cc. of cottonseed oil.
25 Gm. of potassium hydroxide.
100 cc. of glycerin.
15 cc. of alcohol.
A standard thermometer for determining the titer of fatty acids (see U.S.P.).

Procedure. 1. "Heat 75 cc. of glycerin-potassium hydroxide solution (made by dissolving 25 Gm. of potassium hydroxide in 100 cc. of glycerin) to 150° in an 800-cc. beaker, and add 50 cc. of the clarified fat, melted if necessary. Heat the mixture for 15 min. with frequent stirring, but do not allow the temperature to rise above 150°. When saponification is complete, the mixture is homogeneous, with no particles clinging to the beaker at the meniscus."

The oil is saponified by the KOH with the formation of the potassium salts of the fatty acids and glycerin. See Saponification Value (page 280) for the reactions that occur.

2. "Pour the contents of the beaker into 500 cc. of nearly boiling water in an 800-cc. beaker or casserole, add slowly 50 cc. of dilute sulfuric acid (made by adding 1 volume of sulfuric acid to 3 volumes of water), and heat the solution, with frequent stirring, until the fatty acids separate cleanly as a transparent layer. Wash the acids with boiling water until free from sulfuric acid, collect them in a small beaker, and place on a boiling water bath or steam bath until the water has settled and the fatty acids are clear, filter into a dry beaker while hot, and dry for 20 minutes at 100°.

"Place 3 cc. of the dry acids in a test tube and add 15 cc. of alcohol. Heat the solution to boiling and add an equal volume of ammonia T.S. A clear solution should result."

The fatty acids are liberated from their potassium soaps by the sulfuric acid. They are washed to remove potassium sulfate, excess sulfuric acid, and glycerin, and dried to remove the water. If saponification is incomplete, the acids will be contaminated with unsaponified oil, which would alter the solidification temperature of the acids. A turbidity in the alcohol solution indicates incomplete saponification, since the oil is insoluble in the diluted alcohol and the ammonium soap is soluble.

3. "Cool the dry, filtered acids to from 15 to 20 degrees above the expected reading, and transfer to a glass tube 25 mm. in diameter and 100 mm. in length,

the glass being 1 mm. in thickness. By means of a perforated cork fasten the tube in a wide-mouth bottle of clear glass, approximately 70 mm. in diameter and 150 mm. in height. Suspend the thermometer of Type V (see U.S.P.) in the melted acids so that it will serve as a stirrer, cool if necessary, and stir the mass slowly until the mercury remains stationary for 30 seconds. Then allow the thermometer to hang quietly, with the bulb in the center of the acids, and observe the rise of the mercury column. The highest point to which it rises is the Solidification Temperature of the fatty acids."

When the melted acids solidify, the "latent heat of fusion" is liberated, and a rise in temperature occurs, which remains constant for a short time. The fatty acids give a distinct rise in temperature when they solidify, whereas the fats and fatty oils are usually characterized by the fact that they yield a constant temperature, which for some is variable when they solidify.

TABLE 64. OFFICIAL SUBSTANCES WITH CONGEALING TEMPERATURE REQUIREMENTS

Substance	Congealing Temperature, °C.
U.S.P.	
Acetic acid, glacial	(n.l.t.) 15.6
Anise oil	(n.l.t.) 15
Benzyl benzoate	(n.l.t.) 18
Eucalyptol	(n.l.t.) 0
Fatty acids from:	
Cottonseed oil	31 to 35
Olive oil	17 to 26
Peanut oil	26 to 32
Sesame oil	20 to 25
Theobroma oil	45 to 50
Fennel oil	(n.l.t.) 3
Menthol (dl-)	30.5 to 32
Nikethamide	22 to 24
Oleic acid	(n.m.t.) 10
Paraldehyde	(n.l.t.) 11
Phenol	(n.l.t.) 39
N.F.	
Anethole	(n.l.t.) 20
Eucalyptus oil	(n.l.t.) −15.4
Parachlorophenol	42 to 44
Stearic acid	(n.l.t.) 54
Suet, prepared	37 to 40
Undecylenic acid	21 to 22

(n.m.t.) = not more than. (n.l.t.) = not less than.

Questions and Problems

1. The U.S.P. requires that the congealing temperature of phenol should not be below 39°. Why?

2. The determination of the congealing temperature of eucalyptus oil constitutes the official assay process. How does this determination serve as a measure of the purity of the oil?

Boiling and Distilling Ranges. One of the characteristic properties of a liquid is its boiling point. If a substance consists of a single compound, it is generally considered to be pure when it distills at a constant boiling point, under constant pressure. In like manner, the purity of a mixture of substances, such as a volatile oil, may be judged in part by the range of temperature at which the substance distils.

The boiling point of any liquid may be defined as *that temperature at which the vapor pressure of the liquid is equal to the atmospheric pressure.* Where a given temperature is stated as the boiling point of a liquid, the inference is that the corresponding pressure is standard atmospheric pressure, 760 mm., unless otherwise given.

The boiling or distillation range or temperature is *the range of temperatures between which all of a given liquid or a specified percentage of the liquid will distill.*

The apparatus to be used (Fig. 36) and its construction are as follows:

A distilling bulb of from 50- to 60-cc. capacity to the lower part of the neck; the length of the neck should be from 10 to 12 cm. and its internal diameter from 14 to 16 mm. The outlet tube of from 4- to 5-mm. internal diameter should be attached to the neck at approximately its mid-point, forming an angle of 70 to 75 deg. with the lower portion of the neck.

A straight glass condenser with a water jacket from 40 to 60 cm. in length; the distance from the upper end of the water jacket to the neck of the bulb being from 18 to 25 cm.

In order to avoid the necessity for an emergent stem correction, it is advisable that an accurately standardized thermometer of the Anschutz type be used. If such a thermometer is not available, a thermometer used for melting points may be employed, applying the emergent stem correction as described under Melting Point, the length of the emergent stem being measured, in degrees, from the lower end of the stopper to the highest point of the boiling temperature. When placed in position, the lower end of the thermometer should be level with the center of the orifice of the outlet tube. Provide an asbestos board from 12 to 15 cm. square and from 3 to 5 mm. thick, having a circular perforation, located centrally for the reception of the bulb. The edge of the asbestos around the perforation should fit closely to the bulb when the latter is set into it. The size of the perforation should be such that when the bulb is set into it, the portion of the bulb below the upper surface of the asbestos will have a capacity of from 3 to 4 cc.

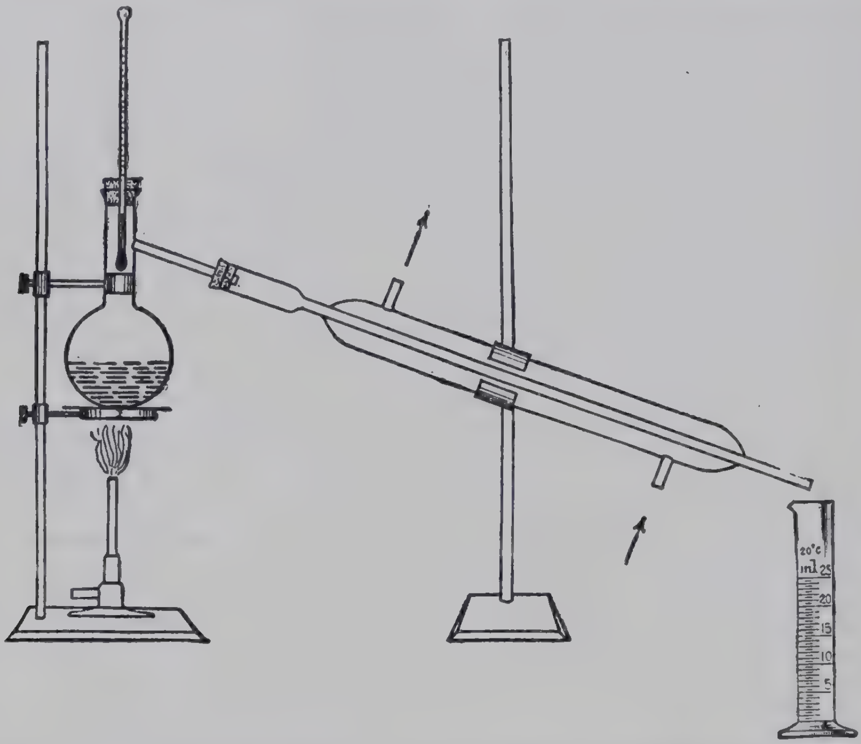

FIG. 36. Apparatus for boiling- and distilling-point determinations.

Exercise 118

Object. To Determine the Boiling Point of Carbon Tetrachloride.

Materials Required. 25 cc. of carbon tetrachloride.
A distillation apparatus as illustrated.

Procedure. Place the asbestos board on a tripod or other suitable support. Introduce into the distilling bulb 25 cc. of the liquid to be tested, insert the thermometer, stand the bulb in an upright position in the perforation of the asbestos board, and connect it with the condenser. Then distill the liquid at the rate of 4 to 5 cc. per min., noting the temperature as soon as 5 drops of the liquid have distilled into the receiver and when the last liquid evaporates from the bottom of the flask or when the specified percentage has distilled over. Correct the reading for any variation in the barometric pressure from the normal (760 mm.) by allowing 0.1° for each 2.7 mm., adding if the pressure is lower, and subtracting if higher than 760 mm.

The temperature obtained when a thermometer is immersed in a boiling liquid is usually greater than the true boiling point of the liquid, because the vapor bubbles form below the surface of the liquid and consequently are at a pressure greater than atmospheric. There is also an appreciable error due, in this case, to superheating of the liquid. When the thermometer is suspended in the vapor of the liquid, however, the bulb becomes coated with a thin film of liquid in contact with its vapor, and superheating is avoided.

It is often best to run a preliminary distillation, of a 25-cc. portion of liquid, prior to the boiling-point determination, in order to ascertain the regulation of heating necessary to cause the entire portion of volatile liquid to distill at the prescribed rate. Superheating of the liquid and consequent bumping can be prevented by placing a few small pieces of porous plate in the distillation flask. The correction for the barometric pressure may be made simply, as illustrated in the following example: If the range of temperature within which 95 per cent of glacial acetic acid distills is 116.5 to 116.9° at 733 mm., the corrected temperatures would be $116.5 + 0.1\left(\frac{760 - 733}{2.7}\right) = 117.5$, and $116.9 + 0.1\left(\frac{760 - 733}{2.7}\right) = 117.9$. The boiling point of the acid should then be reported as $\text{b.p.}_{733} =$ 117.5 to 117.9° cor., the subscript 733 indicating the barometric pressure at which the determination was made, and the abbreviation "cor." indicating that the boiling point as reported has been corrected to standard pressure. If a long-stemmed thermometer is used in the determination, a further correction for the emergent stem must be made, as explained under Melting Point.

A second method is given in the Pharmacopoeia for use with liquids for which the permissible range in boiling temperature exceeds 5°. The following is a description of the apparatus required and the procedure to be followed:

A 200-cc. distilling bulb with an outlet tube at approximately the center of the neck, forming an angle of from 70 to 75 deg., with the lower end of the neck. The length of the neck is from 10 to 12 cm. and its inside diameter is from 18 to 24 mm. The length of the outlet tube is from 10 to 12 cm. and its inside diameter is from 5 to 6 mm.

A straight glass condenser, a thermometer, and an asbestos board are required as in Method I. The diameter of the perforation in the asbestos board is 50 mm.

Place the distilling bulb in an upright position in the perforation in the asbestos board and connect it with the condenser. The outlet tube is to extend from 25 to 35 mm. into the condenser beyond the connecting stopper.

Measure 100 cc. of the liquid to be tested, using a cylinder having 1-cc. graduations. Note the temperature of the liquid and transfer it as completely as possible to the distilling bulb. Use this cylinder as the receiver for the distillate without rinsing out any of the adhering liquid. Insert the thermometer through a stopper in the position described in Method I, and heat with a Bunsen burner protected from drafts until the liquid begins to boil, then distill at the rate of from 4 to 5 cc. per minute, collecting the distillate coming over between the temperatures specified in the text. Bring the distillate to the same temperature at which the liquid was originally measured, and note the volume. Correct the temperature reading for barometric pressure and apply the emergent stem correction as described in Method I.

Liquids that begin to distill below 80° are cooled to 10 or 15° before measuring the 100 cc. for the test. The end of the condensing tube is fitted with an adapter bent at a suitable angle, and the end of the adapter is passed through a stopper inserted into the receiving cylinder. The stopper has a small perforation to permit the exit of air. The receiving cylinder is kept immersed in ice to within 2.5 cm. of its height during the distillation.

When boiling points are given with a range instead of with a single

TABLE 65. OFFICIAL SUBSTANCES WITH BOILING-POINT RANGES

Substance	Boiling-point range, °C.*
U.S.P.	
Amphetamine	200 to 203
Amylene hydrate	97 to 103
Butopyronoxyl	256 to 270†
Cresol	195 to 205†
Dimercaprol	122 (15 mm.) or 116 (10 mm.)
Dimethyl phthalate	278 to 285‡
Ethohexadiol	240 to 250‡
Eucalyptol	174 to 177
Eugenol	250 to 255‡
Glyceryl triacetate	257 to 260‡
Paraldehyde	120 to 126
Petroleum benzin	35 to 80
Phenol, liquefied	Not above 182
Propylene glycol	185 to 195
Tetrachloroethylene	118 to 122†
Trichloroethylene	86 to 88
N.F.	
Acetone	55.5 to 57
Anethole	231 to 237
Benzyl alcohol	202.5 to 206.5§
Carbon tetrachloride	76 to 78
Cetyl alcohol	316 to 336§
Creosote	203 to 220§
Ethyl acetate	76 to 77.5
Guaiacol (liquid)	200 to 210¶
Guaiacol (solid)	204 to 206
Isopropyl alcohol	81 to 83
Oleyl alcohol	305 to 370†
Pine needle oil (dwarf)	Below 165
Pine oil	200 to 225‡
Turpentine oil	154 to 170†

* The temperature between which the substance completely distills at atmospheric pressure unless otherwise specified.

† (n.l.t.) 90 per cent distills between the specified temperatures.

‡ (n.l.t.) 95 per cent.

§ (n.l.t.) 94 per cent.

¶ (n.l.t.) 85 per cent.

figure, the figures are to be regarded as inclusive. The boiling points of such substances as acetone, ether, ethyl oxide, alcohol, and glacial acetic acid should be regarded as informative rather than as criteria of identity and purity, since only approximate boiling points are given. Definite requirements relative to the per cent of a substance that should distill within a certain range of temperature and above or below a certain temperature are given in the official compendiums as follows: 85 per cent of creosote from creosote carbonate distills at 200 to 220°, 90 per cent of creosote distills at 203 to 220°, 90 per cent of cresol distills at 195 to 205°, 90 per cent of turpentine oil and of rectified turpentine oil distills at 154 to 170°, and 85 per cent of liquid guaiacol distills at 200 to 210°C. It is required that the volatile oil separated from copaiba by steam distillation should not boil below 250°, that less than 10 per cent of dwarf pine needle oil should distill below 165°, and that the boiling point of liquefied phenol should not rise above 182° when distilled.

Those official substances for which a definite range of boiling points is specified and within which practically all the substance or a specified proportion (per cent) of the substance should distill, are given in Table 65.

Questions and Problems

1. Define the terms *boiling range* and *distilling range.*

2. How is the distillation range of the creosote in creosote carbonate directed to be determined in the National Formulary?

3. If ether contained alcohol as an impurity, would the boiling point of the ether be raised or lowered?

4. A sample of carbon tetrachloride is found to distill between 76.8 and 77.9° at 664 mm. Calculate the corrected distilling range of the carbon tetrachloride.

5. Consult the appended table and list those substances the boiling points of which should be determined by Method II of the U.S.P.

6. Which is more pure as indicated by the required boiling-point ranges, ethyl acetate of the N.F. or ethyl acetate reagent of the U.S.P.?

Chapter 22

HYDROGEN ION CONCENTRATION, pH, AND POTENTIOMETRIC TITRATIONS

Many of the compounds that are pharmaceutically important are acids, bases, or salts. In aqueous or hydroalcoholic solution they tend, in great or small degree, to dissociate into their respective ions. Volumes of published information bear witness to the fact that these ions and especially the hydrogen ion concentration affect all kinds of chemical interactions. The concentration and activity of hydrogen ions in a solution influence the concentrations of the anions, cations, and undissociated molecules present in the solution. These factors, in turn, often affect the stability, therapeutic activity, and pharmaceutical elegance of medicaments in aqueous or hydroalcoholic solutions. The importance of the hydrogen ion concentration, usually expressed in terms of pH, indicates the need for simple and accurate methods for measurement of this value.

Acid-Base Equilibrium and pH. The dissociation of acids may be represented by the type description:

$$AH \rightleftharpoons H^+ + A^-$$

In a similar manner the dissociation of substituted ammoniums may be represented:

$$BH^+ \rightleftharpoons B + H^+$$

The dissociation of the metallic hydroxides may be represented:

$$BOH \rightleftharpoons B^+ + OH^-$$

For the purpose of this discussion, salts, certain acids, and the hydroxides of the alkali metals are assumed to be completely dissociated in dilute aqueous solution.

At equilibrium the ordinary mass action equation will hold as a first approximation for weak acids

$$\frac{[H^+][A^-]}{[HA]} = K_a \tag{1}$$

$$\frac{[A^-]}{[HA]} = \frac{K_a}{[H^+]} \tag{2}$$

where the brackets indicate molar concentration of each ionic or molecular species.

The constant, K_a, is the dissociation constant of the acid. The value of K_a is markedly different for various acids, and for the same acid it is appreciably affected by the presence and concentrations of various ions in solution. The ratio $[A^-]/[HA]$ in Eq. (2) is seen to depend upon the value of K_a and the concentration of hydrogen ions. Assuming that K_a remains constant, the ratio $[A^-]/[HA]$ can be changed only by changing the value of $[H^+]$. This can be accomplished by the addition of a strongly dissociated acid, which increases $[H^+]$, or the addition of a strongly dissociated base, which decreases the value of $[H^+]$.

Furthermore, the ratio $[A^-]/[HA]$ may be changed by the addition of the acid itself or its salt, but not directly as the ratio of these added quantities, for $[H^+]$ will have been changed also.

The magnitudes of $[H^+]$ vary enormously, *i.e.*, from one to one ten-millionth squared or 10^{-14}. The desirability of an exponential or logarithmic scale of expression is at once apparent. The system generally adopted is that devised by Sørenson, *viz.*, the use of the symbol pH, which is defined as *the negative logarithm of the hydrogen ion concentration* (see page 87). Hence:

$$\text{pH} = -\log [H^+] \quad \text{or} \quad \log \frac{1}{[H^+]} \tag{3}$$

The relation of pH to $[H^+]$ may be seen in Table 66.

The logarithmic nature of this system should be borne in mind constantly in considering changes in hydrogen ion concentration and its connotation in terms of pH as illustrated in Table 67.

pH Measurements. Two basic methods for the determination of pH are generally employed: (1) a colorimetric method and (2) a potentiometric method. Each of these two methods will be discussed.

TABLE 66

pH	Normality, hydrogen ions	Normality, hydroxyl ions
0	1	10^{-14}
1	10^{-1}	10^{-13}
2	10^{-2}	10^{-12}
3	10^{-3}	10^{-11}
4	10^{-4}	10^{-10}
5	10^{-5}	10^{-9}
6	10^{-6}	10^{-8}
Neutral point 7	10^{-7}	10^{-7}
8	10^{-8}	10^{-6}
9	10^{-9}	10^{-5}
10	10^{-10}	10^{-4}
11	10^{-11}	10^{-3}
12	10^{-12}	10^{-2}
13	10^{-13}	10^{-1}
14	10^{-14}	1

TABLE 67

$[H^+]$	pH
Times Increased or Decreased	Corresponding Change
10	1.00
5	0.70
2	0.30
1.5	0.18
1.1	0.04
1.05	0.02
1.023	0.01

Potentiometric Methods. The potentiometric method involves the measurement of the difference of e.m.f. between two half cells, the one a reference and the other the hydrogen ion responsive element. When an acid or an alkali is dissolved in water, the characteristic properties of the solution obtained are due to the hydrogen and hydroxyl ions formed by the dissociation of the acid or base. The effective acidity or alkalinity depends on the extent of dissociation of the acid or base in solution and is independent of the total acidity or alkalinity. In the volumetric estimations of acids and alkalies, the total acidity or alkalinity is determined. In measuring the effective acidity or alkalinity (the strength of the acid or base) of a solution, however, it is necessary to determine the hydrogen ion concentration.

The hydrogen ion concentration of an aqueous solution may be determined by measuring the voltage developed between two electrodes of special character immersed in the solution by means of a potentiometer, since the voltage developed between the electrodes depends directly on the concentration of hydrogen ions in the solution. The measurement of the voltage produced between the electrodes is equivalent, therefore, to a determination of the hydrogen ion concentration and to the determination of the effective acidity or alkalinity of the solution.

The electrodes employed in the determination of hydrogen ion concentration must be of such character that one of them will develop a potential that varies according to the concentration of hydrogen ions, and the other must have a constant potential unaffected by the hydrogen ion concentration. The choice of the reference half cell (electrode) involves several considerations including reproducibility, permanency, ease of preparation, and the temperature coefficient. The saturated calomel electrode is the most practical for all-around use and is the most popular. This electrode contains a permanent charge of calomel (Hg_2Cl_2) and mercury in a protective inner tube and the outer body tube of the electrode contains saturated potassium chloride solution, which maintains electrolytic connection between the electrode assembly and the solution under measurement through a ground-glass sleeve at the lower end of the tube. Another type of arrangement for the calomel electrode is shown in Fig. 37.

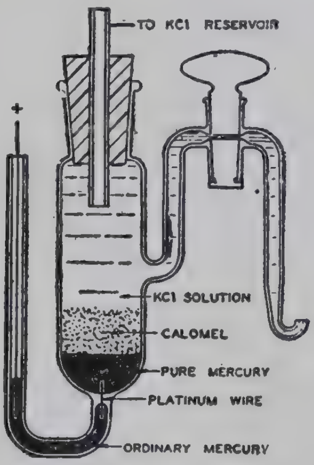

Fig. 37. Calomel electrode.

The hydrogen ion responsive electrode may be one of several different kinds, including the hydrogen gas electrode which has been the ultimate standard of reference, the quinhydrone electrode, metal-metal ion electrodes such as the antimony electrode, the platinum electrode, and the glass electrode. Although several possible combinations may be used, methods employing a glass electrode and a calomel reference electrode are preferred because of simplicity and accuracy in operation.

The electrometric apparatus for measuring the pH may be either the "laboratory" (potentiometric balance) or the "industrial" (direct-reading) type. The principal parts of these apparatus are the same in both types, and, aside from differences in construction due to the different methods of indicating pH, they essentially differ only in the degree of sensitiveness.

The Glass Electrode (Industrial Type Electrometric Apparatus). It has been found that a very thin membrane of special glass separating two solutions gives potentials that depend directly on the hydrogen ion concentrations. The glass electrode (Fig. 38) consists of a glass electrode bulb and an internal reference electrode.

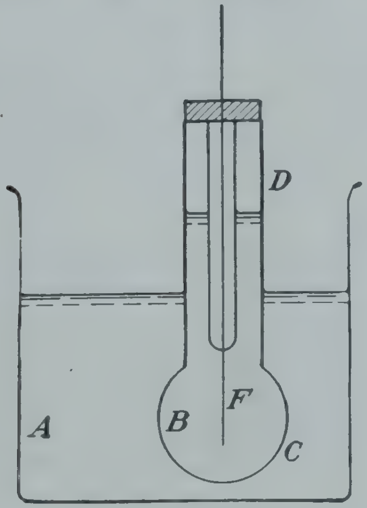

FIG. 38. A glass electrode. A thin glass bulb *C* is blown at the end of a glass tube *D*. Hydrochloric acid, 0.1*N*, or less is placed in the tube with the internal reference electrode *F*. The internal electrode either is silver-coated with silver chloride or may be made of platinum.

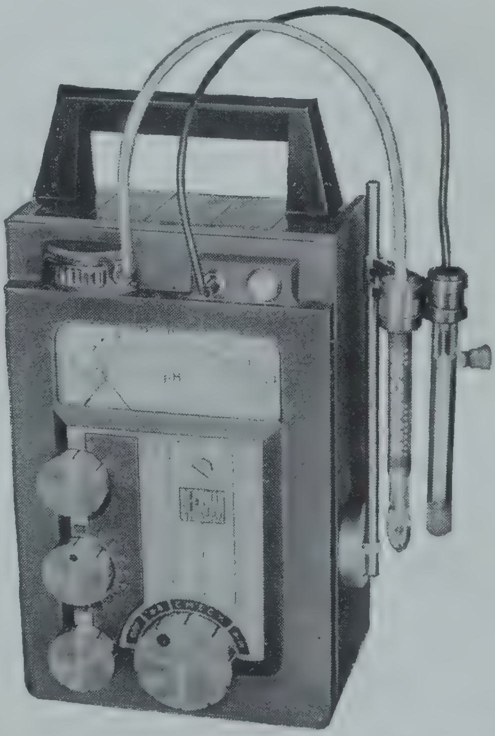

FIG. 39. An industrial type electrometric pH meter.

When the glass membrane is mounted in a cell with the calomel electrode, the voltage is a function of the difference in hydrogen ion activity except for a small "asymmetry potential" due to the glass. A sensitive electrometer or an amplifier of the vacuum tube type is required to measure the voltages, because the resistance of the glass diaphragm is so great that the ordinary galvanometer is inaccurate.

A glass electrode pH meter of the type illustrated in Fig. 39 is desirable for most pharmaceutical work. Instruments of this type are furnished with complete directions for their preparation, operation, and maintenance. Under ordinary conditions, pH values between 1 and 9.6 can be determined with an accuracy of 0.1 pH unit. Above pH 9.6, buffer solutions having approximately the same positive ion concentration as the unknowns being tested should be used to calibrate the instrument.

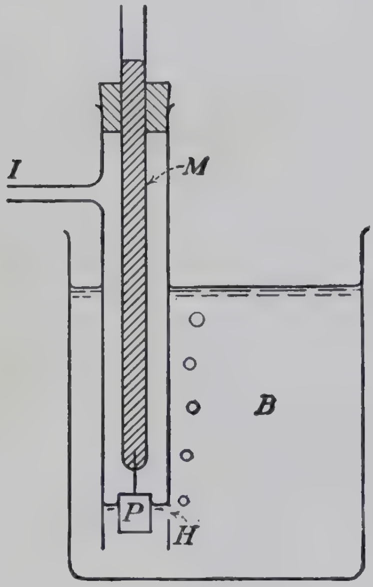

FIG. 40. A hydrogen electrode. (*I*) inlet for stream of hydrogen; (*M*) mercury; (*P*) platinum foil, plated with platinum black; (*H*) hole for escape of hydrogen; (*B*) beaker with solution whose pH is to be determined.

The Hydrogen Electrode (Laboratory Type Electrometric Apparatus). The hydrogen electrode consists of a small square of platinum foil attached to a short piece of platinum wire sealed into and extending through one end of a glass tube. Mercury is poured into the open end of this tube to cover the platinum wire in the bottom so that the electrode may be connected with the potentiometer by dipping a wire into the mercury. This electrode element is placed in another tube of larger diameter by means of a side tube through which purified hydrogen gas is conducted to the electrode when it is immersed in a solution. Holes in the side of this outer tube at the level of the electrode element permit the hydrogen gas to escape without forcing the solution completely out of contact with the platinum.

When the platinum tip of this electrode is coated with platinum black (platinum in a finely divided state), the platinum black absorbs and holds a relatively large volume of the gas. Consequently, when an electrode prepared in this manner is saturated with hydrogen gas and immersed in a solution containing hydrogen ions, it behaves as a hydrogen electrode and develops a potential that is definitely related to the hydrogen ion concentration of the solution.

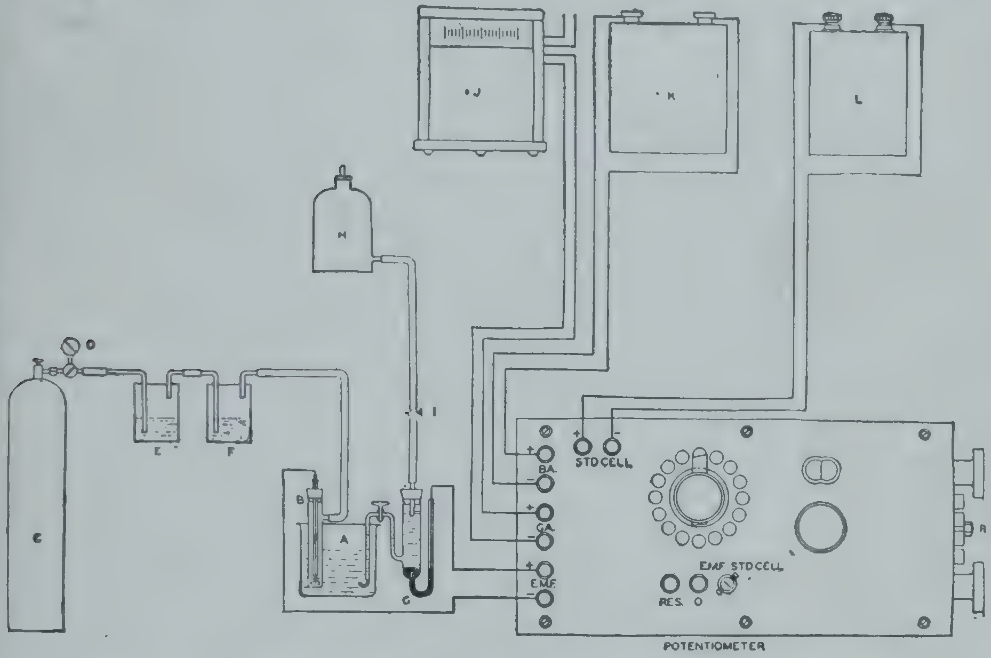

Fig. 41. Scheme of assembly for hydrogen ion measurements.

A "laboratory" arrangement utilizing a hydrogen electrode and a student potentiometer is shown in Fig. 41. *A* is a beaker of 50- to 200-cc. capacity containing the solution being investigated. *B* is the hydrogen electrode. *C* is a cylinder of compressed, electrolytic hydrogen equipped with a pressure-reducing valve *D*. The gas supply is passed through a solution of pyrogallol in potassium hydroxide contained in the gas-washing bottle *E* and then through distilled water contained in the second gas-washing bottle *F* and finally into the hydrogen electrode vessel. The hydrogen gas may be generated from zinc and sulfuric acid, but in this case, the hydrogen should be washed by passing it through successive gas-washing bottles containing alkaline permanganate, alkaline pyrogallol, sulfuric acid, and water to remove arsenic, hydrogen sulfide, etc. All the connections in the gas train should be tight to exclude the entrance of air. Pure gum rubber tubing, 5-mm. bore and of medium wall, should be used for the connections. *H* is an aspirator bottle containing a reserve supply of saturated potassium chloride–calomel solution connected to the calomel electrode vessel so that the side arm of the latter can be flushed out at frequent intervals and before each new determination. The flow of solution from the aspirator bottle is controlled by the pinchcock *I*.

The voltage-measuring equipment consists of a potentiometer, by means of which the voltage produced by the hydrogen and calomel electrodes dipping into the solution is measured by establishing a known voltage in opposition and exactly equal to that of the electrodes; a storage cell, or battery, *K* to supply a constant source of current; a galvanometer *J* which shows when the voltage developed at the electrodes is exactly counterbalanced against the voltage of the potentiometer; and a standard cell *L* to establish known voltage conditions in the potentiometer circuit.

Employing the hydrogen and calomel electrodes at 20° and a potentiometer to measure the voltage, the pH may be calculated by the following equation:

$$\text{pH} = \frac{E - 0.2488}{0.0581}$$

where E is the potential difference expressed in volts between the two electrodes.

Standard Buffer Solutions for Calibrating pH Electrode Assemblies. Buffer solutions must be available for calibrating glass electrode assemblies. These solutions must be stored in bottles of chemically resistant glass and must be prepared from salts prepared for use either singly or in combination as pH standards. Buffer salts of requisite purity can be obtained from the National Bureau of Standards, Washington, D.C. The salts must be dried for 1 hr. at 105° before use, except in the case of borate, which must be used as the decahydrate.

The following solutions are primary standards for calibrating the embly and great care must be exercised in their preparation.

Standard 0.1N hydrochloric acid solution, pH 1.10 at 25°. Prepare 0.1N hydrochloric acid from reagent-grade hydrochloric acid, determine its normality and adjust, if necessary, to 1.0 ± 0.0005N.

Standard 0.05M acid potassium phthalate buffer, pH 4.01 at 25°. Dissolve 10.21 ± 0.05 Gm. of acid potassium phthalate in sufficient water to make 1,000 cc. of solution.

Standard 0.025M phosphate buffer, pH 6.86 at 25°. Dissolve 3.402 ± 0.001 Gm. of potassium dihydrogen phosphate and 3.519 ± 0.001 Gm. of disodium hydrogen phosphate in sufficient water to make 1,000 cc. of solution.

TABLE 68. pH VALUES FOR STANDARD BUFFER SOLUTIONS FROM 0 TO 60°C.

Temperature, °C.	0.1M hydrochloric acid, pH	0.05M phthalate, pH	0.025M phosphate, pH	0.01M borate, pH
0	1.10	4.01	6.98	9.46
5	1.10	4.01	6.95	9.39
10	1.10	4.00	6.92	9.33
15	1.10	4.00	6.90	9.27
20	1.10	4.00	6.88	9.22
25	1.10	4.01	6.86	9.18
30	1.10	4.01	6.85	9.14
35	1.10	4.02	6.84	9.10
40	1.10	4.03	6.84	9.07
45	1.10	4.04	6.83	9.04
50	1.11	4.06	6.83	9.01
55	1.11	4.08	6.84	8.99
60	1.11	4.10	6.84	8.96

Standard 0.01M borate buffer, pH 9.18 at 25°. Dissolve 3.71 ± 0.01 Gm. of decahydrated sodium borate in sufficient water to make 1,000 cc. of solution. Protect the bottle containing this solution from atmospheric carbon dioxide and keep stoppered at all times except when actually in use.

Fresh buffer solutions must be prepared at intervals not exceeding 3 months. The pH values of these buffers at various temperatures are given in Table 68.

Exercise 119

Object. To determine the pH of Isotonic Sodium Chloride Solution.

Materials Required. 10 or 15 cc. of the above solution.

An "industrial" (direct-reading) type or a "laboratory" (potentiometric balance) type pH meter.

A wash bottle.

Standard phthalate and phosphate buffers.

A number of industrial-type pH meters are available. Some of these are line-operated and some are battery-operated, and the electrodes differ somewhat in their physical appearance; however, the basic principle of their operation, previously described, as depending on a glass and a calomel electrode, is the same, and all are satisfactory for the usual pH measurement. In the "laboratory" type meter (see Fig. 41) a hydrogen or a quinhydrone electrode may be used in combination with the calomel electrode. The quinhydrone functions well in acid but not in alkaline solution.

Procedure. 1. Calibration of the Apparatus. Set the temperature knob, if one is provided, to correspond to the proper temperature, turn the instrument on, allow to warm up thoroughly, and bring to electrical balance by carefully following the manufacturer's instructions. Wash the glass electrode thoroughly with water. If the calomel electrode is of the enclosed or pencil type in which contact of the saturated potassium chloride solution with the buffer solution is made through a capillary, wash it in the same manner and at the same time as the glass electrode. If, on the other hand, the calomel electrode is of the type in which the saturated potassium chloride solution has free access to the buffer solution, flush the old junction with water and form a fresh one. It is suggested that the electrodes be given at least five washings with approximately 15 cc. each with a stream of water from a wash bottle followed by two additional washings of approximately 50 cc. each from the mouthpiece of the wash bottle. The sample cup should be similarly washed and shaken to remove the excess drops of water.

In bringing the meter to electrical balance, the voltage of the potentiometer is exactly balanced against that of the standard cell, the voltage of which is known. Balance is indicated when the galvanometer index does not move upon tapping the galvanometer key. Do not hold the galvanometer key down to note the deflection of the index, but tap it firmly and quickly.

2. Fill the sample cup with standard phthalate buffer and immerse the electrodes in the cup. If the "laboratory" type instrument is used, set the dial to read the pH value of the standardizing buffer. Engage the operating button, and rotate the zero-control, sometimes called the asymmetry-potential, knob until the meter is brought to balance. If the "industrial" type of meter is used, engage the operating button and rotate the zero-control knob until the reading of the dial corresponds to the known pH of the standard buffer. Repeat the process using additional portions of the standard buffer until a reproducibility of ±0.02 unit for the "laboratory" or ±0.05 unit for the "industrial" type is reached. Wash the electrodes as described previously.

The second standard buffer selected from the table for the calibration of the apparatus should correspond as closely as possible to the anticipated value for the solution whose pH is to be determined. Repeat the above process but do not disturb the setting of the zero-control knob obtained by use of the first standard buffer. The apparatus is judged to be operating satisfactorily if the reading obtained for the second standard buffer agrees to within ±0.02 unit (for "laboratory" meters) with the known value for that buffer. Wash the electrodes as before. The meter is now considered ready for the determination of the pH of the solution to be tested.

The apparatus must always be calibrated with two buffers to check the linearity of the response of the electrode at different pH values and to detect a faulty glass electrode. The hydrochloric acid and the borate buffer offer the widest difference in pH for this purpose. The standardization of the apparatus with only a single buffer may be completely erroneous because of the wide range of the zero-control knob. The presence of a faulty electrode will be detected by failure to obtain a reasonably correct value (±0.02 unit) for the pH of the second buffer when the meter has been standardized in terms of the first. A cracked electrode will yield pH values which are essentially the same for both buffers. If the difference between the known and the observed pH values for the second buffer exceeds ±0.02, another glass electrode should be substituted. If the difference persists, prepare fresh standard buffer solutions.

The apparatus should be calibrated every time it is used, if only an occasional pH determination is made, and at short convenient intervals, for example, every fifth sample, in a long series of measurements. Perform the calibration with standard buffers at both the beginning and the end of the measurements. Particular care should be taken to keep the electrodes free from deposits of foreign matter.

If the pH of the solution to be tested is more alkaline than 9, it is essential that the type of glass electrode which has a negligible alkali error in the region of the test be used. Such electrodes are usually designated as the "low-alkali-error" type, and the manufacturer's instructions should be carefully followed in their use.

3. Determination of pH of the solution being tested. After the meter has been calibrated with two standard buffer solutions, thoroughly wash the electrodes and the sample cup. Fill the cup with a portion of the solution to be tested and obtain a preliminary value for the pH. In general, this value will drift and is regarded as an approximation. Subsequent readings taken on additional portions of the same solution will yield successively more constant pH values. In the case of solutions which are well buffered, three portions may be sufficient to yield pH values which are reproducible to ±0.02 unit and which show drifts of less than ±0.02 unit in 1 or 2 min. In the case of very dilute or unbuffered solutions, as many as six portions of the test solution may be required, and the pH values may continue to drift and be reproducible to only ±0.05 unit. Record the pH of the solution.

If a precision of greater than 0.1 pH unit is desired, the temperature of the standard buffers, the glass and calomel electrodes, and the test solutions must be within 2° of one another, and the electrodes, buffers, test solutions, and wash water must be kept at the temperature of measurement for at least 2 hr. prior to making the measurement in order to reduce to a negligible value the effects of thermal or electrical hysteresis of the electrodes.

The term pH is expressed generally by an integer and two significant decimals. The reliability of the value of the figures in the second decimal place is dependent largely on the method employed in carrying out the determination. For official purposes, the expression is given with one decimal only. For practically all purposes, this degree of accuracy is considered sufficient. However, the method described is capable of a higher degree of accuracy.

The pH of distilled water is measured by first calibrating the apparatus with standard phthalate buffer and making measurements of the pH of the distilled water, and then recalibrating the apparatus with standard borate buffer and making a second set of measurements of the pH of the distilled water. In this manner two values will result, the first of which will be slightly more acid and the second slightly more alkaline than the true pH of the water. The two values, however, should check to within 0.1 unit and the average should be reported. The "flow-type" electrodes may also be used.

The pH of solutions which are only partially aqueous in character are approximated in the manner described above. Report the values so obtained as *apparent* pH values. It must be clearly recognized that, when a pH meter is standardized in terms of an aqueous buffer and then used to measure the "pH" of a nonaqueous solution or suspension, the ionization constant of the acid or base, the dielectric constant of the medium, the liquid-junction potential (which may give rise to errors of approximately 1 pH unit), and the hydrogen ion response of the glass electrode are all changed. For these reasons the values so obtained must be reported as *apparent* pH values. This is particularly important for hydroalcoholic solutions.

Potentiometric Titrations. The analyst for many years has depended on his ability to perceive indicator color changes as the end point in titrimetric procedures. Several factors influence the accuracy of such indicator titrations, including (1) lighting conditions; (2) color or turbidity of the solution; (3) color blindness; (4) fatigue of the operator; (5) pH range of the indicators used. Of the several methods which have been devised to overcome indicator end points, the potentiometric method is the most important.

The potentiometric method of determining end points, just as in the measurement of pH, depends upon the measurement of the difference in potential between two dissimilar electrodes. The same electrodes used in measurement of pH are applicable to potentiometric titrations.

The difference in potential between two electrodes may be changed by adding a substance to the solution to bring about any of the following chemical reactions: (1) acid-base reaction (neutralization); (2) oxidation-reduction; (3) precipitation; or (4) complex-ion formation.

The potentiometric end-point determination is made possible by the fact that as titration proceeds there is a rapid change at the end point in the concentration of the substance being titrated or of the titrant. This change in concentration causes a rapid change in the potential of the electrode assembly. The end point, then, is reached when the greatest relative change in potential is produced by a small

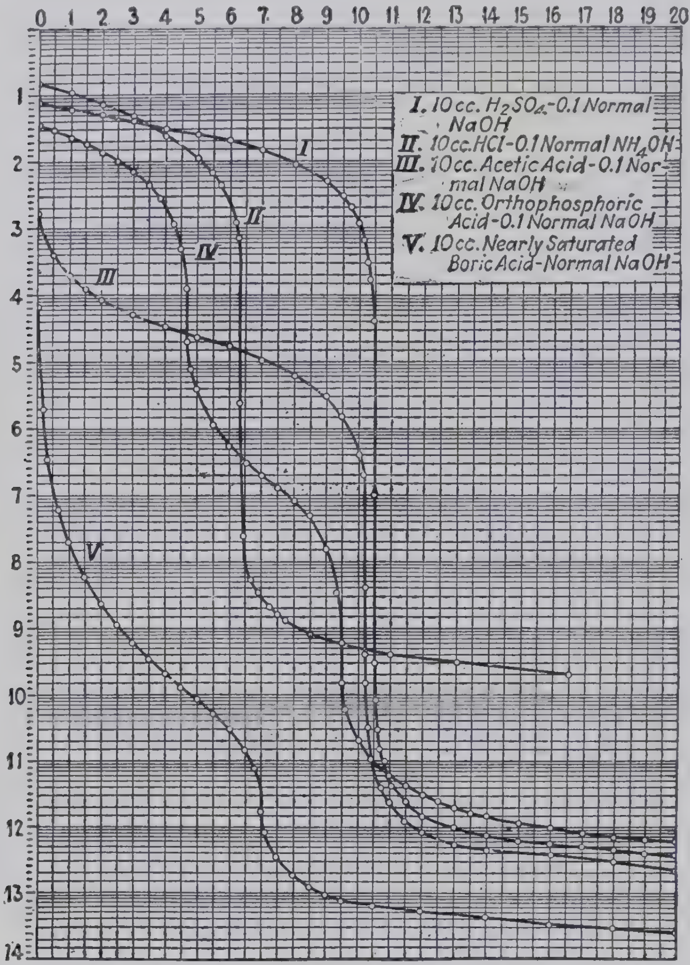

FIG. 42. Characteristic titration curves. Vertical scale shows pH values and the horizontal scale shows cubic centimeters of titrant.

addition of titrant. The sharper the potential change for a given addition, the more exactly may the end point be determined.

To determine the end point of a given titration procedure, cubic centimeters of titrant are plotted on the abscissa against voltage or pH on the ordinate. The shape of such a curve is dependent on the chemical nature and quantity of the reactants only and is independent of any salts in the solution which do not react with the titrant.

The end-point potential is indicated by the inflection of the curve. Some typical titration curves are illustrated in Fig. 42.

Exercise 120

Object. To Determine the End Point of Hydrochloric Acid Titrated with Sodium Hydroxide Potentiometrically.

Materials Required. An "industrial" or "laboratory" type pH meter.
A wash bottle.
Standard buffers.
25 cc. of 0.1*N* hydrochloric acid.
0.1*N* sodium hydroxide.

Procedure. 1. Calibrate the pH apparatus as described in Exercise 119, using two separate buffer solutions.

2. Remove the buffer from the titration vessel, wash the electrodes with distilled water, and, if a closed calomel electrode is not used, flush a small quantity of potassium chloride through the side arm of the electrode. Place 25 cc. of 0.1*N* HCl in a 200-cc. titration beaker and dilute it with about 80 cc. of distilled water. Determine the pH or the voltage by immersing the electrodes in the acid solution. Accurately measure about 20 cc. of 0.1*N* sodium hydroxide solution into the titration beaker and again determine the voltage or pH. Add successively four accurately measured 1-cc. portions of 0.1*N* sodium hydroxide and determine the voltage or pH after the addition of each cubic centimeter. Continue to add portions of sodium hydroxide solution, dropwise, and determine the voltage or pH after the addition of each portion until 26 cc. have been added. Add 5 cc. more of 0.1*N* alkali and determine the voltage or pH. Plot a curve using cubic centimeters of 0.1*N* sodium hydroxide solution as abscissas and voltage or pH readings as ordinates, and determine the end point of titration from the curve. Calculate the hydrogen ion concentration at the end point.

If the normality of both the acid and alkali solutions used is not known, it is advisable to run a preliminary test, using a suitable indicator to determine the end point, so that the equivalent point determined potentiometrically will not be overrun. As alkali is run into the acid in the titration, it will be noticed that the voltage changes slowly at first, more or less gradually as the equivalent point is approached, very rapidly with respect to very small amounts of sodium hydroxide at the equivalent point, and then very slowly again as an excess of alkali solution is added.

The only official potentiometric titration assay is the Karl Fischer titration for water. The method involves the titration of the sample in reagent methanol with the Karl Fischer reagent which consists of iodine, sulfur dioxide, pyridine, and methanol. The reactions of this reagent with water may be expressed by the following equations:

$$H_2O + I_2 + SO_2 + 3C_5H_5N \longrightarrow 2C_5H_5N.HI + C_5H_5N.SO_3$$
$$C_5H_5N.SO_3 + CH_3OH \longrightarrow C_5H_5NH.SO_4CH_3$$

For many substances the end point can be detected visually by the change from a brownish yellow to an amber color. However, for colored substances or when the end point is not very sharply defined, the potentiometric method is to be preferred since there is a definite sharp potential change at the end point. Figure 43 gives a typical Karl Fischer titration assembly. Several instruments based on this principle and designed especially for water determinations using the Karl Fischer reagent are now available commercially. Consult the U.S.P. for more specific information concerning this method of water determination.

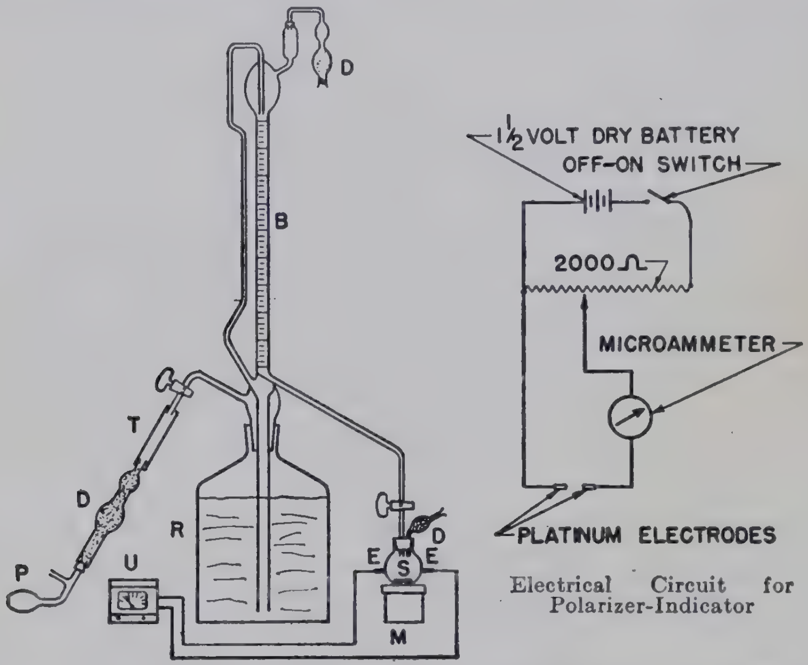

FIG. 43. Automatic burette and titration assembly for Karl Fischer water determination. (*B*) burette; (*D*) desiccant; (*E*) platinum electrodes; (*M*) electric magnetic stirring apparatus; (*P*) pressure bulb; (*R*) reservoir for Karl Fischer solution; (*S*) plastic or glass-coated stirring bar; (*T*) rubber tubing; (*U*) polarizer-indicator.

Colorimetric Methods for Determining pH. The hydrogen ion concentration of solutions can also be determined colorimetrically. The potentiometric method is more accurate, in general, and is to be preferred, even though it requires a more elaborate apparatus and more experience on the part of the analyst; however, for convenience both procedures are official in the U.S.P.

A number of colorimetric methods for the approximate determination of hydrogen ion concentration have been developed. The most generally used of these involve the matching of the color obtained when a definite quantity of indicator is added to a definite volume of the liquid being tested, with that of one of a series of standard buffer solutions differing by about 0.2 pH unit. The volume of the unknown and of the standard buffer solution must be the same, both of them must contain the same amount of indicator solution, and the colors must be matched by viewing through the same depth or thickness of solution. Except in a few cases, the limit of accuracy attainable by the colorimetric method does not exceed 0.1 pH unit.

The principle of the colorimetric method depends upon the assumption that an indicator in the same concentration will exhibit the same transition shade in different aqueous solutions if the hydrogen ion concentrations of the solutions are the same. Having then a series of buffer solutions of definite hydrogen ion concentrations, comparisons can be made between the shades of color produced in the buffer solutions and that produced by the same indicator in the solution to be tested.

As previously stated, the K_a of an acid is affected by the ionic strength of the solution, likewise the K_a of indicators is also affected. This influences the color transition range of the indicator. The potentiometric method measures the activity of the hydrogen ions (H^+), and to obtain the same value by the colorimetric method in solutions of various ionic strengths, a correction, known as the *salt-error correction*, must be employed. In addition, the kinds of ions present in solution and their precipitating influence on the indicator affect the accuracy of the colorimetric method. For official purposes, where the complexity of the medicaments makes a simple application of these corrections impossible, the corrections are ignored.

pH Indicators and Preparation of Their Solutions. The indicators used for colorimetric pH determinations are either weakly acid or weakly basic. Most of the indicators used for this purpose, however, such as the phthaleins and sulfonated phthaleins, behave like weak acids.

The usual concentration of the indicator solution is 0.05 per cent. From 0.1 to 0.2 cc. of the indicator solution is generally used for 10 cc. of the liquid being examined.

Solutions of indicators of the basic type and of the phthaleins are prepared by dissolving them in alcohol. In preparing solutions of indicators containing an acid group, this group must first be neutralized with sodium hydroxide. The procedure is as follows:

One-tenth (0.100) Gm. of the indicator is triturated in an agate mortar with the volume of $0.05N$ sodium hydroxide specified in the following table, or with its equivalent of $0.02N$ sodium hydroxide. When the indicator has dissolved, the solution is diluted with carbon-dioxide-free distilled water to make 200 cc. (0.05 per cent).

The solutions should be kept in stoppered bottles and protected from light.

TABLE 69. pH INDICATORS

Indicator	pH range	Molecular weight	Color change	Solvent
Methyl yellow	2.9 to 4.0	225	Red–yellow	Alcohol
Bromophenol blue	3.0 to 4.6	669	Yellow–blue	3.0 cc. of $0.05N$ NaOH
Bromocresol green	3.8 to 5.4	699	Yellow–blue	2.8 cc. of $0.05N$ NaOH
Methyl red	4.2 to 6.3	269	Red–yellow	7.4 cc. of $0.05N$ NaOH
Bromocresol purple	5.2 to 6.8	540	Yellow–purple	3.7 cc. of $0.05N$ NaOH
Bromothymol blue	6.0 to 7.6	624	Yellow–blue	3.2 cc. of $0.05N$ NaOH
Phenol red	6.8 to 8.4	354	Yellow–red	5.7 cc. of $0.05N$ NaOH
Thymol blue	8.0 to 9.6	466	Yellow–blue	4.3 cc. of $0.05N$ NaOH
Thymolphthalein	9.3 to 10.5	430	Colorless–blue	Alcohol

Directions for the pH Measurements. In the determination of an unknown pH value, a suitable indicator must first be found. Only indicators that show an intermediate color between the extreme acid and alkaline colors can be used.

The first step in the choice of a suitable indicator is the determination of the approximate pH value of the solution under investigation. A few simple tests will usually supply the necessary information. Add a drop or two of phenolphthalein T.S. to a small portion of the solution. If the indicator remains colorless, the pH of the solution is less than 8.4. A second test is conducted in the same manner, using methyl orange T.S. as the indicator. If the solution assumes the alkaline color (yellow), the pH of the solution is greater than 4.4 and lies somewhere between 4.4 and 8.0. A few more tests with methyl red (pH interval 4.2 to 6.3), bromothymol blue (6.0 to 7.6), and phenol red (6.8 to 8.4) will give a close enough approximation of the pH value to show which indicator may be successfully used in the determination.

Instead of testing small amounts of the liquid with indicator solutions, the spot method, using indicator papers, may be substituted. Also, by using a universal indicator in place of the several indicator solutions suggested above, some time may be saved. A so-called "universal" or "mixed" indicator of this type can be prepared as follows: Dissolve 0.1 Gm. of phenolphthalein, 0.2 Gm. of methyl red, 0.3 Gm. of dimethylaminoazobenzene, 0.4 Gm. of bromothymol blue, and 0.5 Gm. of thymol blue in 500 cc. of absolute alcohol and then add sufficient sodium hydroxide to produce a yellow color. When 0.1 to 0.5 cc. of this mixed indicator is added to an unknown solution, the color produced indicates the approximate pH of the solution as follows: Red color = pH 2; orange color = pH 4; yellow color = pH 6; green color = pH 8; and blue color = pH 10.

When the approximate pH value has been determined and a suitable indicator agreed upon, 2- to 10-cc. portions of the unknown solution (depending upon the amount of the liquid available), are transferred to a hard, resistant-glass test tube approximately 15 cm. long and 1.5 cm. bore, and a measured amount of the indicator solution added. As a rule, 0.10 to 0.20 cc. of a 0.05 per cent indicator solution, added from a 1-cc. pipette, graduated to 0.01 cc. per 10 cc. of the solution being tested, constitutes a proper indicator concentration.

Transfer from 4 to 6 portions of the buffer solutions, the pH values of which overlap that of the unknown solution, to test tubes and treat in exactly the same way as the solution being analyzed. The same amounts of indicator must be added to the unknown and to the buffer solutions. It is also essential that the test tubes used be of the quality and type already indicated. The color of the unknown solution is then compared with the colors of the buffer solutions, and the pH value of the solution thus determined.

In judging the colors, observe them against a white background, with the light transmitted through the whole length of the tube. A suitable colorimeter may also be used, although it is not necessary in routine work. A sufficient number of reference solutions must be taken so that the color of the unknown falls between two of the series, differing by not more than 0.20 pH. The pH of the unknown can thus be easily approximated to within 0.1 and with practice to 0.05. With buffer solutions differing by 0.1 pH unit or less, the experimental error can be reduced to about 0.02 pH.

Other colorimetric methods capable of the same or a greater degree of accuracy than that officially required may be employed at the discretion of the operator.

The dissociation constants of indicators hold for aqueous solutions only. Alcohol changes this value and, therefore, for alcoholic solutions a correction factor must be used or the potentiometric method employed.

Hydrogen Ion Concentration (pH) of Some Official Substances. The hydrogen ion concentrations, expressed as pH, of the official substances in Table 70 are not intended to be construed as a means for the determination of the purity of these substances. In practice, variations from these figures may frequently be found, as a slight excess of acid or base is, in many instances, desirable and even necessary to ensure stability and other qualities in connection with the use of these substances.

Questions and Problems

1. Enumerate several instances in the description of official products where such terms as *slightly acid*, *alkaline to litmus*, etc., could be replaced by definite limits of p*H* to advantage.

2. What advantages does the colorimetric method have that are not possessed by the hydrogen electrode method of hydrogen ion determination?

3. Name several classes of pharmaceutical preparations of which the pH cannot be determined colorimetrically.

TABLE 70. pH OF SOME OFFICIAL SUBSTANCES

Substance	Concentration	pH
U.S.P.		
Aluminum phosphate gel	....	6.0 to 7.2
Aluminum sulfate	1 in 20	(n.l.t.) 2.9
Anticoagulant acid citrate dextrose solution	....	4.5 to 5.5
Bentonite	1 in 50 (suspension)	9 to 10
Bismuth potassium tartrate injection	....	6.0 to 9.0
Caffeine and sodium benzoate injection	....	6.5 to 8.5
Calcium gluconate injection	....	6.0 to 8.2
Carbachol injection	....	5.0 to 7.0
Cherry juice	....	3.0 to 4.0
Congo red injection	....	7.0 to 9.0
Dextrose and sodium chloride injection	....	4.0 to 6.0
Dextrose injection	....	4.0 to 6.0
Dihydromorphinone hydrochloride injection	....	4.0 to 5.5
Emetine hydrochloride injection	....	3.0 to 4.0
Ephedrine sulfate injection	....	4.5 to 7.0
Epinephrine injection	....	2.5 to 5
Ergotamine tartrate injection	....	4 to 6
Heparin sodium	1 in 100	6 to 7.5
Insulin injection	....	2.5 to 3.5
Insulin injection, globin zinc	....	3.4 to 3.8
Insulin injection, protamine zinc	....	7.1 to 7.4
Meperidine hydrochloride injection	....	4.5 to 6
Meralluride injection	....	7 to 8.5
Mercurophylline injection	....	7.5 to 9.0
Mersalyl and theophylline injection	....	7.5 to 8.5
Methacholine chloride injection	....	4 to 6
Methiodal sodium injection	....	6 to 8
Morphine injection	....	(n.l.t.) 3
Naphazoline hydrochloride	1 in 100	(n.l.t.) 6.1
Nicotinamide injection	....	5.0 to 7.0
Nikethamide	1 in 3	6.0 to 6.5
Nikethamide injection	....	5.5 to 6.5
Papaverine hydrochloride injection	....	(n.l.t.) 3
Penicillin G potassium	3 in 100	5.0 to 7.5
Penicillin G sodium	3 in 100	5.0 to 7.5
Pentobarbital sodium injection	....	8 to 10
Pentylenetetrazol injection	....	6 to 8.5

TABLE 70. pH OF SOME OFFICIAL SUBSTANCES. *(Continued)*

Substance	Concentration	pH
U.S.P.		
Phenobarbital sodium injection		8.5 to 10
Phenolsulfonphthalein injection		6.0 to 7.5
Phenylephrine hydrochloride injection		3 to 5
Piperocaine hydrochloride injection		4 to 6
Polyethylene glycol 4000	1 in 20	4.5 to 7.5
Polysorbate 80	1 in 20	5 to 7
Procaine hydrochloride and epinephrine injection		3.3 to 5.5
Procaine hydrochloride injection		3.3 to 5.5
Quinacrine hydrochloride	1 in 100	About 4.5
Quinine and urethan injection		5.0 to 7.0
Raspberry juice		2.7 to 3.8
Ringer's solution		5.0 to 7.5
Ringer's solution, lactated		6.5 to 7.5
Silver nitrate ophthalmic solution		5 to 6
Sodium ascorbate injection		5.5 to 7.0
Sodium chloride solution, isotonic		4.5 to 7.0
Sodium lactate injection	0.2*M*	6.0 to 7.3
Stibophen injection		5.5 to 7.5
Sulfadiazine sodium injection		8.5 to 10.5
Sulfamerazine sodium injection		8.5 to 10.5
Sulfathiazole sodium injection		8.5 to 10.5
Sulfobromophthalein sodium injection		5 to 6.5
Suramin sodium	1 in 100	5.5 to 7.0
Suramin sodium, sterile	1 in 100	5.5 to 7.0
Thiamine hydrochloride injection		(n.l.t.) 2.5
Thiamine mononitrate	1 in 50	6.0 to 7.5
N.F.		
Amobarbital sodium	1 in 20	9.70 to 10.00
Calcium levulinate	1 in 10	7.0 to 8.5
Dibucaine hydrochloride	1 in 50	5.0 to 6.0
Proflavine dihydrochloride	1 in 1,000	2.5 to 3.0
Proflavine sulfate	1 in 1,000	2.5 to 3.0
Sodium dehydrocholate		9.5 to 10.5
Sodium thiosulfate injection		8.0 to 9.5
Thimerosal	1 in 100	6.7

(n.l.t.) = not less than.

4. Explain how the end point in the titration of alkaloids with acids might be determined potentiometrically.

5. Referring to the characteristic potentiometric titration curves, what indicator could be used for titrating a weak acid with a strong base; a strong acid with a strong base? Explain your choice of indicator.

6. Why is it desirable to have an exponential or logarithmic scale for expression of hydrogen ion concentration?

7. What does the choice of the reference electrode involve?

8. Explain the basic principle involved in potentiometric titrations and the determination of pH using the potentiometric method.

9. Consult the recent journal literature (during two preceding years) and briefly summarize the articles treating of the application of hydrogen ion concentration measurements to pharmaceutical products.

10. In the potentiometric titration of 25 cc. of $0.2680N$ H_2SO_4 with NaOH solution, using the hydrogen electrode and a saturated calomel electrode, the following are values in millivolts for the corresponding volumes of NaOH: 0.0 cc. = 370.0; 5 cc. = 378.1; 10 cc. = 388.0; 15.0 cc. = 397.6; 20 cc. = 406.0; 25.0 cc. = 420.2; 28.0 cc. = 460.1; 30.0 cc. = 516.1; 30.5 cc. = 690.5; 31 cc. = 860.1; 35.0 cc. = 949.5; 40 cc. = 965.9; 45 cc. = 982.2; 50 cc. = 992.1. Plot the millivolts of electrode potential as ordinates and the cubic centimeters of NaOH as abscissas. Determine the pH value at the end point from the curve and calculate the normality of the sodium hydroxide solution.

Chapter 23

COLORIMETRY, SPECTROPHOTOMETRY, FLUOROPHOTOMETRY, NEPHELOMETRY, AND TURBIDIMETRY

Optical methods of analysis may be defined as those methods of analysis which depend upon a change in the character or the amount of radiant energy as it passes through a substance. Radiant energy in a discussion of optical methods of analysis is taken to mean ultraviolet, visible, and infrared regions of the radiant-energy spectrum. This is approximately in the range of 200 to 2,500 mμ wavelengths. Light is taken to mean only the visible range of the spectrum (400 to 750 mμ).

There are several important optical methods of analysis, including (1) spectrochemical; (2) spectrophotometry; (3) colorimetry; (4) fluorophotometry; (5) turbidimetry; (6) nephelometry; (7) refractometry; (8) polarimetry. Polarimetry and refractometry are optical methods in which the character of the light is changed. These two methods are sufficiently different from other methods of optical analysis that they are taken up under separate chapter headings. Spectrochemical analyses, the determination of the wavelength of absorption or emission bands, are not used in the official methods and therefore do not come within the scope of this text.

Spectrophotometry, colorimetry, fluorophotometry, turbidimetry, and nephelometry are all optical methods depending upon a change in the amount of radiant energy transmitted and are sufficiently similar in method and application that they are considered together in this chapter.

Changes in the amount of radiant energy transmitted may be due to three factors: (1) absorption; (2) reflection; (3) fluorescence. Those methods based on the absorption of radiant energy are termed *absorptimetric* methods and the process is known as *absorptimetry.* Colorimetry and spectrophotometry are absorptimetric methods.

Measurements based on the reflection of radiant energy are termed *nephelometric* methods and the process is termed *nephelometry*. Measurements based on the fluorescence of radiant energy are called *fluorometric* methods of analysis and the process is known as *fluorophotometry*. Absorptimetric methods are the most important of the official optical methods and are taken up first.

COLORIMETRY

The term *colorimetry* is limited to the estimation of the concentration of colored materials in solution by matching the color of the unknown solution with that of a standard. The use of color as a means of determining the amount of a given constituent in a substance has been utilized for many years in analytical work. It is only in recent times, however, that the methods have been extended and refined. Colorimetric methods of analysis generally consist in adding a reagent to a solution of the substance being tested so that a color is produced, and comparing the intensity of color in the product with that produced by adding the same reagent to a solution of the substance of known concentration. Frequently, they are rapid and accurate; however, their greatest advantage is their extreme sensitivity, which makes it possible to determine very small amounts of substance.

Colorimetric Apparatus. The simplest type of apparatus for colorimetric measurements consists of the Nessler tubes and rack (Fig. 44). The tubes have parallel sides and flat bottoms and are graduated to 50 and 100 cc. Nessler tubes should be of the same diameter, but frequently they are not. Consequently, they should be compared for equality of distance between the 50- and 100-cc. graduation marks and only those which agree should be selected. The use of this apparatus is illustrated in the following exercise.

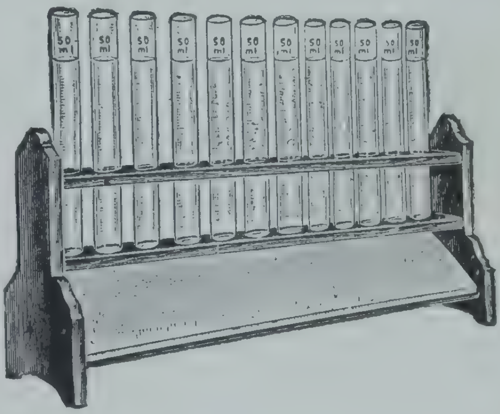

FIG. 44. Nessler apparatus.

Exercise 121

Object. To Determine the Ammonia Content of Water.

Materials Required. Natural water (use tap water).

Ammonium chloride solution. Dissolve 0.3141 Gm. of pure ammonium chloride in ammonia-free water and dilute to 100 cc. Dilute 10 cc. of this solution to 1,000 cc. with ammonia-free water. Each cubic centimeter of the resulting solution contains the equivalent of 0.01 mg. of NH_3.

Rochelle salt solution. Dissolve 5 Gm. of potassium and sodium tartrate in 10 cc. of water.

Nessler's reagent. Dissolve 10 Gm. of potassium iodide in 10 cc. of water, and add slowly, with stirring, a saturated solution of mercury bichloride until a slight red precipitate remains undissolved. To this mixture add 30 Gm. of potassium hydroxide. After solution has taken place, add 1 cc. more of the saturated solution of mercury bichloride. Dilute with water to 200 cc. Allow the precipitate to settle and draw off the clear liquid. A 2-cc. portion of this reagent, when added to 50 cc. of water containing 0.05 mg. of ammonia, produces at once a yellowish-brown coloration.

Ammonia-free water. Test the distilled water in the laboratory by placing 50 cc. in a Nessler tube previously washed several times with the distilled water and add 2 cc. of Nessler's reagent. Cover the tube and allow it to stand for 5 min. If the color produced after standing is more intense than that of the Nessler's reagent immediately after dilution, the water must be purified. If purification is necessary, add 1 Gm. of potassium permanganate to 1,000 cc. of the distilled water, distill, and when the distillate does not give a test for ammonia with Nessler's reagent, collect the water in a clean bottle, previously rinsed with a portion of the ammonia-free distillate.

12 Nessler tubes.

A Nessler tube stand.

Procedure. Place 50 cc. of the water to be tested in one Nessler tube. Place 0.1, 0.2, 0.3, 0.4, 0.5, etc., cc. of the ammonium chloride solution in the other eleven Nessler tubes and add ammonia-free distilled water up to the 50-cc. mark. Add 2 cc. of Rochelle salt solution and 2 cc. of Nessler's reagent to each of the tubes. Mix the contents of the tubes thoroughly and place them in the stand. While looking vertically down through the solution, vary the standard color solutions until the color of the unknown matches one or lies between two of them. Calculate the milligrams per liter of ammonia in the sample of water tested.

When comparing the intensity of color in the tubes, place them in the stand and adjust the position of the latter in front of a window, so that light from the opaque reflector will pass upward through the tubes.

Calcium and magnesium are precipitated from aqueous solution by Nessler's reagent. The Rochelle salt is added to prevent this.

Nessler's solution reacts with the ammonia and ammonium salts to form an orange-colored compound of the composition $HgO.Hg(NH_2)I$. When Nessler's reagent is added to solutions containing more than a few milligrams of ammonia per 100 cc., a distinct orange-colored precipitate is formed, and very small concentrations of ammonia, such as 1 part in 1,000,000, produce a distinct yellow-to-brown coloration.

The number of milligrams of ammonia per liter of water may be calculated as follows: Assume that the color produced in the sample exactly matches that in the tube containing 0.5 cc. of the ammonium chloride solution. Since each cubic centimeter of ammonium chloride solution contains the equivalent of 0.01 mg. of NH_3 per cubic centimeter, 0.5 cc. will contain the equivalent of 0.005 mg. per cubic centimeter or 5.0 mg. per liter. The sample of water must contain the same amount or 5 mg. per 1,000 cc. equivalent to 5 parts of ammonia in 1,000,000 parts of water.

The U.S.P. calls for the use of Nessler tubes in the determination of the maximum fluorine content in dibasic calcium phosphate using alizarinsulfonate T.S. as the color-forming agent and also as a means for checking the color requirements of glycerin. The N.F. uses Nessler tubes to determine the color standard requirements of carmine solution and cochineal solution. Simple color comparisons are used in the official tests for carbonizable substances. These qualitative tests are based upon matching the color developed when a substance is treated with sulfuric

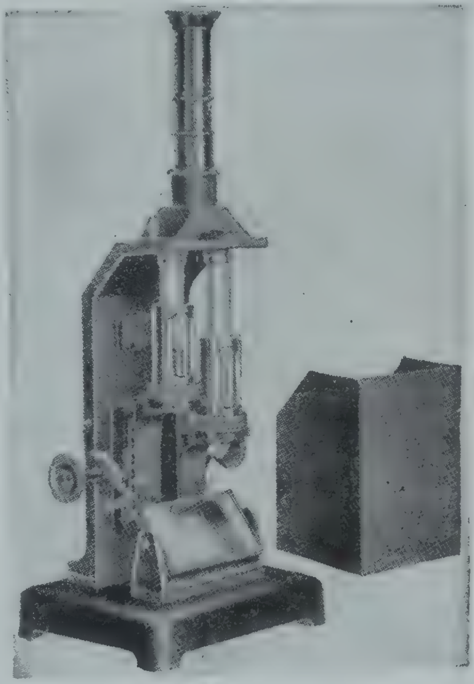

FIG. 45. Duboscq colorimeter.

acid with that of various standard matching fluids. (See the U.S.P.)

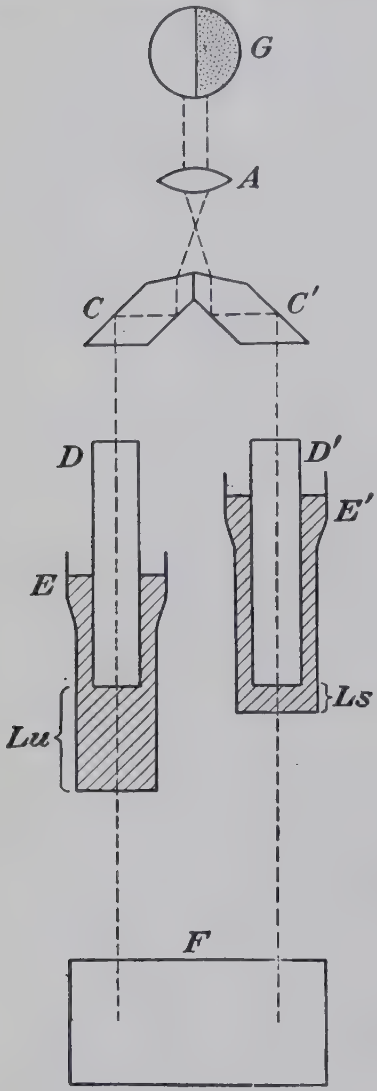

FIG. 46. Path of light through a Duboscq colorimeter. (*A*) lens in the telescope; (*C,C′*) glass prisms; (*D,D′*) fixed glass plungers; (*E,E′*) movable cups; (*F*) mirror; (*G*) adjacent halves of the field; (*Ls*) depth of standard solution through which the light passes; (*Lu*) depth of unknown solution through which the light passes.

Colorimeters. A number of colorimeters have been developed for rapid and precise colorimetric measurements. The *Duboscq colorimeter* (Figs. 45 and 46) is a device of this type, and since it is generally used, its construction and use will be described here. The essential parts of the instrument consist of plungers, cups, a reflecting mirror, a compound prism, an observation microscope, and adjustments. Two prisms of optical glass, hexagonal in form, matched for color, and with optically plane and parallel ends, are attached to the frame of the instrument by threaded metal adapters. Two detachable cups, in which the liquids under examination are placed, are threaded on movable stages beneath the plungers. The cups are made of glass fitted into metal casings and have bottoms made of optically inactive glass disks. An adjustable mirror with two reflecting surfaces is so placed that light can be reflected through the cups. A compound prism is built in above the plungers in a dustproof housing in such a manner as to bring the light beams through each cup to a common axis, light from one cup illuminating one half of a circular field and light from the other cup illuminating the other half. An observation microscope, by which the observer views both fields of illumination with one eye, is focused on the two fields. Adjustments are provided whereby the cups may be raised or lowered, the level of each cup being shown on a scale and vernier.

Exercise 122

Object. To Determine the Amount of Epinephrine Hydrochloride in Solution of Epinephrine Hydrochloride.

Materials Required. 0.050 Gm. of pure epinephrine.
0.1*N* hydrochloric acid.
10 cc. of 1 per cent solution of potassium iodate.
Commercial epinephrine hydrochloride solution 1 to 1,000.
A Duboscq colorimeter.

Procedure. 1. Place 0.050 Gm. of pure epinephrine in a 50-cc. volumetric flask, add 0.5 cc. of 0.1*N* HCl, and dilute the solution with sufficient distilled water to make 50 cc.

This standard solution of epinephrine hydrochloride containing 1 part of epinephrine to 1,000 parts of solution must be prepared freshly as needed.

2. Place 20 cc. of distilled water in a 50-cc. Erlenmeyer flask, add 5 cc. of 1 per cent potassium iodate solution and 0.25 cc. of 0.1*N* hydrochloric acid, and warm the solution to 38°. Then add exactly 0.5 cc. of the standard epinephrine solution and heat the resulting solution at about 38° for exactly 15 min. Prepare another acid solution of potassium iodate in the same manner, warm it to 38°, add exactly 0.5 cc. of the sample of epinephrine hydrochloride to be tested, and heat this solution at 38° for exactly 15 min.

The greatest intensity of color is produced after the epinephrine has been heated for about 10 min. at 38°. It is important that the unknown and the standard solution be heated at the same temperature for the same length of time.

3. Adjust the colorimeter, place it before an ample source of light, preferably diffused daylight, and regulate the mirror so that the two halves of the field are of the same color and equal brightness. Fill one of the cups about one-half full with the known solution and the other cup about one-half full of the unknown. Replace the cups in the instrument and raise them until the plungers come in contact with the bases. Adjust the liquid depth in the cup containing the known solution to any convenient level, preferably to an exact number of millimeters. Raise or lower the cup containing the unknown liquid until both halves of the field are of the same color and intensity. Take three independent readings of the instrument with the cups set at different levels. Calculate the percentage of epinephrine in the unknown solution.

In adjusting the colorimeter, make certain that the plungers and cups are fitted tightly. Raise the cups until the plungers come in contact with the cup bases. The verniers should then read zero. The best source of illumination is diffused daylight obtained by placing the colorimeter before a north window, but that produced by an electric colorimeter lamp may be used. The colorimeter cups should never be filled much more than one-half full; otherwise their liquid contents may overflow when the cups are raised. After placing the known and the unknown liquids in the colorimeter cups, raise the latter until the plungers come in contact with the cup bases to force any bubbles of air out from beneath the ends of the plungers.

In taking readings, set the level of the cup containing the known liquid at some definite position, *e.g.*, 15 mm., and regulate the level of the cup containing the unknown liquid until a color match is secured. Repeat this procedure with the cup containing the known liquid set at 20- and 25-mm. readings. When the two halves of the field of the colorimeter are of the same color and intensity, the color intensities of the two solutions are inversely proportional to each other as the depth of the solution compared. If C_1 and D_1 are the respective concentration and depth of the known solution, and C_2 and D_2 are the corresponding symbols for the unknown, then $C_1 \times D_1 = C_2 \times D_2$. If $C_1 = 1{,}000$ mg. per liter, $D_1 = 20$ mm., and $D_2 = 25$ mm., then: $1{,}000 \times 20 = 25 \times C_2$, and

$$C_2 = \frac{1{,}000 \times 20}{25} = 800 \text{ mg. per liter}$$

the concentration of the unknown.

Photoelectric colorimeters in which the absorption of light is measured by sensitive photoelectric cells may also be used for this type of determination since these instruments are commercially available.

Questions and Problems

1. Is the principle involved in colorimetric measurements with the Duboscq colorimeter the same as when Nessler tubes are used?

2. What are colorimetric solutions? (See U.S.P.)

3. Look up the official test for the limit of carbonizable impurities in the U.S.P. Are these colorimetric tests?

4. Consult the reference at the end of this chapter, and give several applications of colorimetric methods of analysis to pharmaceutical products.

5. What is meant by optical methods of analysis? Classify such methods.

6. Define absorptimetric methods of analysis and give two such methods.

7. What is meant by radiant energy when speaking of optical methods?

8. Describe in general the possible mechanism of a photoelectric colorimeter. Would the use of such a device give more accurate results? Why?

SPECTROPHOTOMETRY

To be able to understand the basic fundamentals and principles of spectrophotometry, it is important that we review briefly the physics of radiant energy and the definitions and symbols commonly used in this type of analysis.

A fusion of the theories of radiant energy calls for a corpuscular form of energy (quanta) propagated in wave form. From the simple theory of wave motion the length of a complete wave or cycle, from the peak of one wave to the peak of the next, is termed the wavelength and can be accurately measured. The unit of measurement generally used in spectrophotometry is the *millimicron*, $m\mu$, and is equal to 10^{-9} meter. Another unit sometimes referred to is the *angstrom* (A.), which is defined as 1×10^{-8} cm.

The dimensions of radiant-energy waves of importance to spectrophotometry are defined as follows:

Ultraviolet—200 to 400 $m\mu$.
Visible—400 to 750 $m\mu$.
Infrared—750 to 2500 $m\mu$.

Spectrophotometry is the science of measurement of the absorption, by substances, of radiant energy of definite and narrow wavelength range, approximating monochromatic radiation. *Monochromatic radiation* is radiation of a single wavelength. The usual spectral region isolated in the better commercial spectrophotometers ranges from 0.5 to 10 $m\mu$, which approximates monochromatic light.

Photometry is a term applied to a form of spectrophotometry usually employing visible radiation, the incident beam of which is not monochromatic but which is more or less restricted by the use of filters.

Spectrophotometry is based on the relation known as the Lambert-Beer or Bouguer-Beer law, relating incident radiation intensity, transmitted intensity, length of the radiation path, and the concentration of the solution.

The mathematical relationship is

$$I = I_o e^{-hcl}$$

where I = intensity of the transmitted radiation.
I_o = intensity of the incident radiation.
e = base of natural logarithms = 2.7183.
h = extinction coefficient. The extinction coefficient is a constant for any one wavelength and material under investigation.
c = concentration of the solution, usually expressed as grams per liter.
l = length of the radiation path, usually expressed in centimeters

Based on this mathematical relationship, certain terms and symbols have found usage and are applicable to spectrophotometric analyses. The important ones with the symbols used are as follows and are designated as official in the U.S.P.:

Transmittancy, I/I_o (T). The ratio of the transmitted light intensity to the incident light intensity. $T \times 100$ = per cent transmittancy (t).

Absorbency, ($-\log T$) (A). The negative logarithm of the transmittancy, also called optical density.

Concentration, (c). Where related to spectrophotometry, concentrations are expressed in grams of solute per liter of solution.

Specific Absorbency, ($E_{1\text{cm.}}^{1\%}$). The absorbency of a solution containing 1 Gm. per 100 cc. contained in a cell having an absorption path of 1 cm.

Specific Extinction Coefficient, (K). The absorbency of a solution containing 1 Gm. per liter contained in a cell having an absorption path of 1 cm.

Applied to the analysis of solutions, spectrophotometry has more general application than colorimetry because the absorption depends upon wavelength in a complicated manner that is characteristic of the chemical composition of the absorbing substance. Measurement of the absorption at several different wavelengths, therefore, permits identification of the solute as well as determination of its concentration. Measurements may be made over a continuous wavelength range, yielding an absorption *versus* wavelength curve, or the absorption may be determined at a number of discrete isolated wavelengths only.

APPARATUS FOR MEASURING RADIATION ABSORPTION

Several models of instruments suitable for making spectrophotometric and similar types of measurement are available commercially. Fundamentally they are of three classes; (1) filter photometers; (2) spectrophotometers; (3) infrared spectrophotometers. Except for differences in the radiant-energy source, methods of restricting wavelength, and certain refinements, these three instruments are basically the same, providing for a radiant-energy source, one or more absorption cells for holding the sample and reference solutions, and a photometric arrangement for measurement of the intensity of the transmitted energy.

Filter photometers, as the name implies, are designed so that interchangeable color filters are used to furnish more or less narrow bands of wavelengths from a continuous source of radiant energy, usually restricted to the visible range. With this type of instrument, absorption can be measured at only certain discrete wavelengths. The intensity of the radiant energy is usually determined by a photoelectric cell.

Spectrophotometers are instruments designed to provide a continuous wavelength range in the visible ultraviolet and near infrared spectra of definite and narrow width approximating monochromatic light. Such instruments are more versatile in their utilization. An instrument such as the Beckman Quartz Spectrophotometer is a good example. This instrument has a continuous range from 200 to 1,000 mμ isolating spectral regions of from 0.5 to 1.5 mμ. A schematic diagram of the optical system of the Beckman Model D.U. Photoelectric Quartz Spectrophotometer is given in Fig. 47.

Infrared Spectrophotometers are designed for operation in the infrared region only and are at present somewhat limited in their use since such instruments are very expensive and the useful range for most purposes in pharmaceutical procedures includes only the visible and near ultraviolet.

No attempt will be made to give detailed instructions for the operation of spectrophotometers since these are usually supplied by the manufacturers; however, special emphasis is given on the following points.

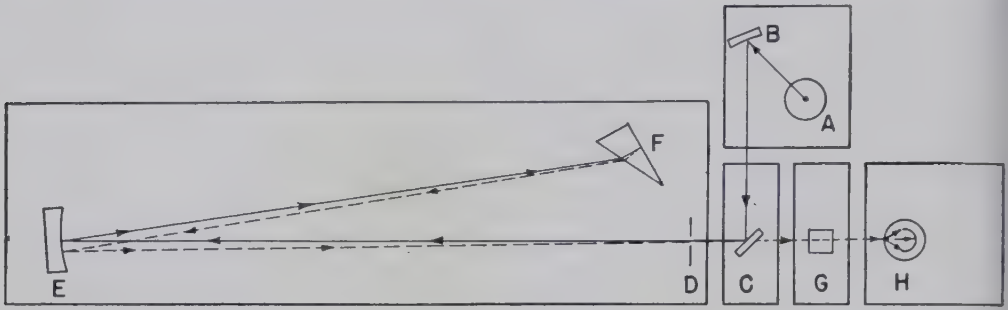

FIG. 47. Schematic diagram of the optical system of the Beckman D.U. spectrophotometer. An image of the light source *A* is focused by the condensing mirror *B* and diagonal mirror *C* on the entrance slit at *D*. The entrance slit is the lower of two slits placed vertically over each other. Light falling on the collimating mirror *E* is rendered parallel and reflected toward the quartz prism *F*. The back surface of the prism is aluminized so that light refracted at the first surface is reflected back through the prism, undergoing further refraction as it emerges from the prism. The collimating mirror focuses the spectrum in the plane of the slits *D* and light of the wavelength for which the prism is set passes out of the monochromator through the exit (upper) slit, through the absorption cell *G* to the phototube *H*.

The instrument must be checked for accuracy of calibration. Where a continuous source is used, attention should be paid to both the wavelength and intensity scales; where a spectral line source is used, only the intensity scale need be checked. For checking the photometric scale, a number of standard glasses are available as well as standard solutions of known transmittancies and absorbencies. For further details regarding a check on both the wavelength and the photometer scales of a spectrophotometer, reference should be made to "Standards for Checking the Calibration of Spectrophotometers (200 to 1000 mμ): Letter Circular LC-929, November 26, 1948, National Bureau of Standards, Department of Commerce, Washington 25, D.C."

Solvents used for dilution usually require special purification, hich is often exacting and different from the requirements for other ses.

Procedure. The general spectrophotometric procedure involves, rst of all, the preparation of a spectral transmittance curve (*S-T* urve) of the solute in question. This curve (see Fig. 48) consists f plotting the per cent transmittancy or absorbency of the solute gainst the wavelength. In this manner one can determine the avelength or series of wavelengths at which maximum absorption ccurs. At these wavelengths the influence of the solute being etermined is at the maximum while interference of other possible onstituents of the solution will be minimized.

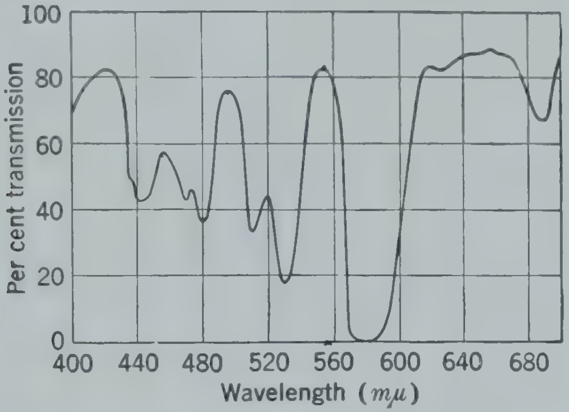

FIG. 48. A typical spectral transmittance (*S-T*) curve.

Having selected the optimum wavelength from the *S-T* curve, a ransmittance-concentration, *T-C*, curve is prepared by plotting per ent transmittance (or absorbency) against a series of known solute oncentrations. Having this curve enables the determination of nknown solute concentrations by merely determining the per cent ransmittance.

Exercise 123

Object. The Assay of Digitoxin.

Materials Required. 20 mg. of digitoxin.
20 mg. U.S.P. digitoxin reference standard.
Trinitrophenol.
Sodium hydroxide solution (1 in 10).
A photoelectric filter photometer or a spectrophotometer.

Procedure. 1. Prepare a standard digitoxin solution. Dissolve 20 mg. of U.S.P. digitoxin reference standard, previously dried at 105° for 1 hr. and accurately weighed, in alcohol to make 100 cc. Preserve under refrigeration in a tight container.

2. Prepare a trinitrophenol solution. On the day of the assay, dissolve 1.8 Gm. of trinitrophenol (calculated on an anhydrous basis) in 50 cc. of alcohol, add 5 cc. of sodium hydroxide solution (1 in 10) and water to make 100 cc.

3. Prepare a standard digitoxin graph as follows: Transfer 2.0-, 3.0-, 4.0-, and 5.0-cc. portions of the Standard solution representing 0.4, 0.6, 0.8, and 1.0 mg. of digitoxin to individual 25-cc. volumetric flasks. Add 15 cc. of the trinitrophenol solution to each flask, dilute to 25 cc. with alcohol, mix thoroughly, and allow to stand for 30 min. Determine the absorbency of each solution in a spectrophotometer or a suitable photoelectric filter photometer, using a filter having a maximum transmission at about 525 mμ, and plot the observed absorbency on the ordinate scale against the corresponding digitoxin concentration on the abscissa scale. Prepare a blank composed of 15 cc. of the trinitrophenol solution, dilute to 25 cc. with alcohol, and use it to determine 0.0 absorbency.

U.S.P. reference standard is used for the preparation of the absorbency-concentration curve, since it is known to be pure digitoxin, and the alcohol solution prepared is stored under refrigeration to prevent decomposition.

Trinitrophenol is added to produce a colored compound which on examination of its *S-T* curve shows maximum radiant-energy absorption at about 525 mμ. This is the reason for selection of this particular wavelength to prepare the absorbency-concentration curve.

The absorbency is determined by setting the instrument at 525 mμ, placing the trinitrophenol blank in an absorption cell in the light path, and setting the instrument to read 0.0 absorbency. The various standard solutions to be tested are then placed in the light path and the absorbency obtained directly from the instrument. These values are used to plot the absorbency-concentration curve.

4. Dissolve 20 mg. of digitoxin, previously dried at 105° for 1 hr. and accurately weighed, in alcohol to make exactly 100 cc. Transfer 4.0 cc. of this solution to a 25-cc. volumetric flask. Add exactly 15 cc. of the same trinitrophenol solution used for preparing the Standard digitoxin graph, dilute to 25 cc. with alcohol, mix thoroughly, and allow to stand for exactly 30 min. Determine the absorbency of this solution, using the same instrument and under the

ame conditions used in preparing the Standard digitoxin graph. From the bserved absorbency, calculate the weight of digitoxin.

The same trinitrophenol solution, instrument, and other conditions are followed as in the preparation of the graph to cancel out ommon errors. The trinitrophenol-digitoxin mixture is allowed to stand for 30 min. to allow for the color reaction to become stabilized. The weight of the digitoxin in the 4-cc. aliquot can be determined directly from the Standard graph.

Questions and Problems

1. Define the terms: (*a*) light; (*b*) radiant energy; (*c*) photometry; (*d*) infrared; (*e*) optical density; (*f*) specific absorbency; (*g*) filter photometer; (*h*) *T-C* curve; (*i*) millimicron; (*j*) absorbency.

2. How does colorimetry differ from spectrophotometry? Which gives rise to more precise measurements? Why?

3. Outline the general procedure for determining a substance spectrophotometrically.

4. Why is a colored compound formed between digitoxin and trinitrophenol in the assay of digitoxin?

5. Give the Lambert-Beer or Bouguer-Beer law and explain the symbols used in the mathematical relationship.

Exercise 124

Object. Assay of the Vitamin A Content of Halibut Liver Oil.

Materials Required. 1 Gm. of halibut liver oil.

Isopropyl alcohol (U.S.P.). Redistill if necessary. This reagent must meet the following requirements for spectral purity: When measured in a 1-cm. quartz cell against distilled water, it must show an absorbency no greater than 0.01 between 350 and 320 mμ and no greater than 0.05 at 300 mμ.

Sodium sulfate, anhydrous, granular.

Phenolphthalein T.S.

Freshly redistilled ether. Discard the first and last 10 per cent portions.

Potassium hydroxide solution, 50 per cent *W/W*.

Antimony trichloride.

Spectrophotometer. An instrument such as the Beckman Quartz Spectrophotometer or one of equal accuracy and sensitivity must be employed. The instrument must be operated using the minimum slit width at optimum sensitivity. Matched quartz absorption cells must be used to hold the sample.

Procedure. 1. Transfer an accurately weighed sample of 0.5 to 1.0 Gm. of halibut liver oil to a saponification flask. Boil under a condenser, in an all-glass apparatus with 30 cc. of alcohol and 3 cc. of potassium hydroxide solution, for 30 min.

To prevent destruction of vitamin A by light during the sampling and analysis, amber or nonactinic glassware must be used unless the assay is carried out under subdued light. Throughout the procedure care must be taken to avoid undue exposure to oxidizing agents: peroxide free reagents and promptness in completing the assay are important. Rubber stoppers and corks may not be used. Other fat-soluble substances occurring with vitamin A interfere with its determination, making some purification necessary. This is accomplished by saponification and extraction.

2. Cool the solution, and add 30 cc. of water. Transfer the resulting solution to a Squibb-type separator, and extract four times with 30-cc. portions of ether. Combine the ether extracts, and wash by swirling gently with 50 cc. of water. Repeat, washing more vigorously until the last washing gives no color with phenolphthalein T.S. Evaporate the ether extract on a water bath to about 50 cc., add 5 to 10 Gm. of anhydrous sodium sulfate, stir, and allow to settle.

The unsaponifiable fraction, containing the vitamin A, of the oil is extracted in the ether and the ether is dried by treating with anhydrous sodium sulfate.

3. Decant into a 100-cc. volumetric flask, and add ether rinsings of the sodium sulfate to make exactly 100 cc., after demonstrating quantitative removal of vitamin A from the sodium sulfate by the addition of a few drops of antimony trichloride solution to the dry residue. Evaporate a 25-cc. aliquot of the ether solution of the unsaponifiable extract to about 5 cc. Without the application of heat, remove the remaining ether in a stream of inert gas or by vacuum. Dissolve the residue in sufficient isopropyl alcohol to give an expected concentration of 8 to 15 U.S.P. Units of Vitamin A per cubic centimeter or such that will give an absorbency in the range 0.4 to 0.8 at 325 mμ. Determine the absorbencies at the wavelengths 310 mμ, 325 mμ, and 334 mμ.

The final solution in isopropyl alcohol should be made immediately after removal of the ether is complete to prevent possible oxidation of vitamin A. In determining the absorbencies one absorption cell of a matched pair is filled with isopropyl alcohol and the second is filled with the sample. The instrument is adjusted to 0.0 absorbency with the solvent followed by determination of the absorbency of the vitamin A sample. The absorption peak of the vitamin A curve is 325 mμ. At this wavelength the absorbency is proportional to the concentration according to Beer's law. Absorbncies at 310 and 334 mμ are taken to correct for extraneous, irrelvant absorbency of the oxidation products of vitamin A.

4. Calculate to three significant places:

$$\text{(corrected)} = 7A_{325} - 2.625A_{310} - 4.375A_{334}$$

$$\frac{\text{(corrected)}}{L \times C} \times 5{,}700 \times \frac{1}{0.3}$$

$$= \text{U.S.P. units of vitamin A per Gm., capsule, or tablet}$$

The absorbencies at 325, 310, and 334 mμ are substituted in the ormula to determine the corrected absorbency at 325 mμ, taking nto consideration the irrelevant absorption. The factors are btained from the general formula for irrelevant absorption[1] as ollows:

$$2.625 = 7\,\frac{(334 - 325)}{(334 - 310)} \qquad \text{and} \qquad 4.375 = 7\left(1 - \frac{334 - 325}{334 - 310}\right)$$

Corrected A is substituted into the second formula, in which

A = corrected absorbency at 325 mμ.

L = length of the light path, cm.

C = amount of sample expressed as grams, capsule, or tablet represented in each 1,000 cc. of the final isopropyl alcohol dilution of the sample under assay.

5,700 = a factor for conversion from spectrophotometric, absorbency, to gravimetric, gram, units.

0.3 = a factor to convert grams to U.S.P. units. Determined from biological potency methods.

Sample Calculation.

Halibut liver oil sample weight = 0.7218.

Saponification sample taken up in 100 cc. ether.

2-cc. aliquot evaporated and diluted to 100 cc. with isopropyl alcohol for spectrophotometric reading.

$$\text{Concentration of spectrophotometric sample} = \frac{0.7218}{50} = 0.01443$$

Gm. per 100 cc. = 0.1443 Gm. per 1,000 cc.

Light path = 1 cm.

Absorbencies found: $A_{310} = 0.481$; $A_{325} = 0.582$; $A_{334} = 0.491$.

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[1] Morton, R. A., and A. L. Stubbs, *Analyst*, **71**, 348, 1946.

Substituting in correction formula:

$$A \text{ (corrected)} = 7(0.582) - 2.625(0.481) - 4.375(0.494) = 0.650$$

Substituting in the final formula:

$$\frac{0.650}{0.1443} \times 5{,}700 \times \frac{1}{0.3} = 85{,}576 \text{ U.S.P. units of vitamin A per Gm.}$$

U.S.P. halibut liver oil contains in each gram not less than 60,000 U.S.P. units of vitamin A.

In addition to using the spectrophotometer for assay purposes, the U.S.P. also uses ultraviolet absorption coefficients ($E_{1\,\text{cm.}}^{1\%}$) for identification tests. Table 71 lists the official limits of these ultraviolet absorption coefficients.

TABLE 71. OFFICIAL LIMITS OF ULTRAVIOLET ABSORPTION COEFFICIENTS

Substance	Solvent	Wave-length, mμ	$E_{1\,\text{cm.}}^{1\%}$
U.S.P.			
Calciferol	Alcohol	265	445 to 485
Chloramphenicol	Water	278	298
7-Dehydrocholesterol (activated)	Alcohol	265	450 to 490
Desoxycorticosterone acetate	Alcohol	240	About 450
Ethisterone	Alcohol	241	480 to 510
Methyltestosterone	Alcohol	241	495 to 530
Progesterone	Alcohol	241	515 to 545
Testosterone	Alcohol	241	520 to 560
Testosterone pellets	Alcohol	241	520 to 560
Testosterone propionate	Alcohol	241	450 to 485

Table 72 lists the official substances assayed using spectrophotometric methods.

Questions and Problems

1. What is another term for absorbency?
2. Why must the reagents like the isopropyl alcohol and the ether used in the vitamin A assay be purified?
3. Why are the oils containing vitamin A saponified before determining absorbency with the spectrophotometer?

4. Matched quartz glass absorption cells are specified in the U.S.P. assay r vitamin A. Why?

5. Could a filter photometer or an infrared photometer be used in the vitamin assay? Why?

6. Look up the reference for the general formula for calculation of irrelevant sorbencies and explain the derivation of the correction equation.

FLUOROPHOTOMETRY

A number of materials can be determined with greater sensitivity nd specificity by fluorophotometric methods than by other optical ethods such as spectrophotometry. However, the method is mited to the analysis of those substances which fluoresce. *Fluorohotometry* consists of the measurement of the intensity of fluoresence of the solute when fluorescence is excited by radiant energy, sually in the ultraviolet region. The general procedure consists of luminating the sample with monochromatic ultraviolet radiant nergy and measuring photoelectrically, at right angles to the incient radiation, the fluorescent light produced in the sample. Calulations are similar to those of spectrophotometry, being based on Modified Beer's law expression. The analysis is so similar to the pectrophotometric type procedure that the same instrument can be sed when suitable accessories are made available by the manufacurer. Fluorophotometric analyses can be made with the Beckman D.U. Spectrophotometer by the use of such attachments.

The method has found most application in the analysis of the itamins and in particular in the analysis of thiamin and riboflavin, which methods are official.

The so-called "thiochrome method" for the analysis of thiamin lepends upon the oxidation of thiamin to thiochrome which fluoresces when exposed to ultraviolet rays in the range from 350 to 400 mμ. Briefly, the procedure consists of acid extraction of the arious forms of thiamin present in the sample, treating the extract with freshly prepared diastatic and phosphorolytic enzyme solution o convert all bound thiamin to its free form, centrifuging and passng the supernatant solution through a *base exchange silicate* to emove interfering substances, eluting the thiamin from the column with hot potassium chloride, oxidizing the thiamin to thiochrome sing alkaline potassium ferricyanide solution and finally determin-

TABLE 72. OFFICIAL SUBSTANCES ASSAYED USING SPECTROPHOTOMETRIC METHODS

Substance	Amount used or sought, mg.	Wavelength, mμ	Official requirement, per cent*
U.S.P.			
Carbachol injection	8	520	$C_6H_{15}ClN_2O_2$ = 90 to 110
Carbachol tablets	8	520	$C_6H_{15}ClN_2O_2$ = 90 to 110
Digitoxin	20	525	$C_{41}H_{64}O_{13}$ = (n.l.t.) 95
Digitoxin injection	2	525	$C_{41}H_{64}O_{13}$ = 90 to 110
Digitoxin tablets	2	525	$C_{41}H_{64}O_{13}$ = 90 to 110
Digoxin injection	4	620	Digoxin = 90 to 110
Digoxin tablets	1	620	Digoxin = 90 to 110
Epinephrine bitartrate	40	530	$C_9H_{13}NO_3.C_4H_6O_6$ = (n.l.t.) 96
Epinephrine in oil injection	10	530	$C_9H_{13}NO_3$ = 90 to 120
Ergonovine maleate injection	2	540 to 560	$C_{19}H_{23}N_3O_2.C_4H_4O_4$ = 90 to 110
Ergonovine maleate tablets	4	540 to 560	$C_{19}H_{23}N_3O_2.C_4H_4O_4$ = 90 to 110
Ergotamine tartrate injection	3	540 to 560	$(C_{33}H_{35}N_5O_5)_2.C_4H_6O_6$ = 90 to 110
Estradiol benzoate injection	1.5 to 10	525, 420	$C_{18}H_{23}O.C_7H_5O_2$ = 90 to 115
Estradiol tablets	2	525, 420	$C_{18}H_{24}O_2$ = 90 to 115
Folic acid	50	550	$C_{19}H_{19}N_7O_6$ = (n.l.t.) 94
Folic acid capsules	50	550	$C_{19}H_{19}N_7O_6$ = 90 to 115
Folic acid tablets	50	550	$C_{19}H_{19}N_7O_6$ = 90 to 115
Halibut liver oil	500 to 1,000	310, 325, 334	(n.l.t.) 60,000 units vitamin A per Gm.
Halibut liver oil capsules	500 to 1,000	310, 325, 334	(n.l.t.) 60,000 units vitamin A per Gm.
Hexavitamin capsules	500 to 1,000	310, 325, 334	(n.l.t.) 5,000 units vitamin A per capsule
Hexavitamin tablets	500 to 1,000	310, 325, 334	(n.l.t.) 5,000 units vitamin A per tablet
Histamine phosphate injection	0.02 to 0.03	460	$C_5H_9N_3.2H_3PO_4$ = 90 to 110
Homatropine methylbromide tablets	10	525	$C_{17}H_{24}BrNO_3$ = 90 to 110
Menadione capsules	25	635	$C_{11}H_8O_2$ = 90 to 120

Menadione injection	25	635	$C_{11}H_8O_2$ = 90 to 120
Nicotinamide capsules	50	500	$C_6H_6N_2O$ = 95 to 110
Nicotinamide injection	50	500	$C_6H_6N_2O$ = 95 to 115
Nicotinamide tablets	100	500	$C_6H_6N_2O$ = 95 to 115
Oleovitamin A	500 to 1,000	310, 325, 334	850 to 1,100 units vitamin A per Gm.
Oleovitamin A capsules	500 to 1,000	310, 325, 334	Oleovitamin A = 95 to 105
Oleovitamin A and D	500 to 1,000	310, 325, 334	850 to 1,100 units vitamin A per Gm.
Oleovitamins A and D, concentrated	500 to 1,000	310, 325, 334	50,000 to 65,000 units vitamin A per Gm.
Oleovitamins A and D capsules, concentrated	500 to 1,000	310, 325, 334	Oleovitamin A = 95 to 105
Ox bile extract	1,000	640 to 680	$C_{24}H_{40}O_5$ = (n.l.t.) 45
Ox bile extract capsules	100	610 to 680	$C_{24}H_{40}O_5$ = (n.l.t.) 10
Ox bile extract tablets	1,000	610 to 680	$C_{24}H_{40}O_5$ = (n.l.t.) 10
Procaine hydrochloride and epinephrine injection	0.15 to 0.25	530	$C_9H_{13}NO_3$ = 90 to 120
Vitamin B_{12}	2	361	Vitamin B_{12} = (n.l.t.) 95
Vitamin B_{12} injection	2	361	Vitamin B_{12} = 90 to 115
N.F.			
Anthralin	100	354, 432	$C_{14}H_{10}O_3$ = (n.l.t.) 95
Anthralin ointment	300	354, 432	$C_{14}H_{10}O_3$ = 90 to 115
Ergot	10,000	590	Ergotoxine = (n.l.t.) 0.15
			Ergonovine = (n.l.t.) 0.01
Ergot, prepared	10,000	590	Ergotoxine = (n.l.t.) 0.15
			Ergonovine = (n.l.t.) 0.01
Rutin	50	362.5, 375.0	$C_{27}H_{30}O_{16}$ = (n.l.t.) 95
			Quercetin = (n.m.t.) 5
Rutin tablets	20	362.5, 375.0	$C_{27}H_{30}O_{16}.3H_2O$ = 92.5 to 107.5

---

* Per cent of the labeled amount unless otherwise indicated.
(n.l.t.) = not less than. (n.m.t.) = not more than.

ing the fluorescence in a suitable fluorophotometer by comparison with the fluorescence obtained from a standard solution of thiamin hydrochloride reference standard.

The fluorometric method for the assay of riboflavin briefly consists of extraction of the riboflavin from the sample with hydrochloric acid (0.1*N*) by autoclaving at 121 to 123° for 30 min., removing interfering substances by precipitation through pH adjustment, followed by oxidation with potassium permanganate, and finally measurement of the fluorescence of the riboflavin in a suitable fluorophotometer as compared to the fluorescence of U.S.P. riboflavin reference standard, taking into consideration the fluorescence of a blank determination.

Questions and Problems

1. To what types of materials is fluorophotometric analysis limited?
2. Explain the phenomenon of fluorescence.
3. How do fluorophotometers differ from spectrophotometers?
4. List the factors on which the intensity of fluorescence depends.

NEPHELOMETRY

Nephelometric measurements afford a means of determining directly the mass or weight of a precipitate in suspension, *i.e.*, without filtering, drying, and weighing. The method commonly used is based upon the measurement of the brightness of the light reflected by a cloud of finely divided particles suspended in a liquid. When other conditions remain constant, the intensity of the reflected light is a function of the amount of suspended particles.

Nephelometric methods of analysis have been applied to a great number of inorganic and organic compounds, such as chlorides, sulfates, ammonia, phosphorus, silver, proteins, fats, volatile oils, and alkaloids. Many substances can be determined quantitatively by nephelometric methods in concentrations of 0.1 to 1 part or less in 1,000,000 parts of water; *e.g.*, 1 part of phosphorus in 330,000,000 parts of water has been determined by this method. Generally, nephelometric methods are limited to measurements of suspensions of substances in low concentration, usually not greater than 100 mg. per liter. Larger amounts of substances can be estimated, however, if properly diluted.

Nephelometric instruments, in general, resemble colorimeters, with the sole major difference that the cells containing the turbid mediums are illuminated at right angles to the direction of observaion. The instrument used for nephelometric measurements may consist of a Duboscq colorimeter fitted with special nephelometer attachments. These attachments include two cups with transparent sides and black bottoms, in place of the colorimeter cups; two tubular metal light shields which surround the cups in such a way that the depth of the liquids illuminated by a light source may be varied; a special nephelometer illuminator; two condensers by means of which a pair of parallel rays are projected horizontally into the cups containing the liquids under examination. Nephelometric determinations preferably should be carried out in a dark room.

The calculations are based on the principle that the turbidities are inversely proportional to the length of the column of liquid. Thus, if C_1 is the concentration of the standard solution, C_2 the concentration of the unknown, L_1 and L_2 the lengths of the columns of liquids in the cups containing the known and unknown solutions, respectively, the concentration of the unknown solution may be found by substituting in the following formula:

$$C_2 = \frac{C_1 \times L_1}{L_2}$$

At present there are no official assays based upon nephelometric procedures.

TURBIDIMETRY

Turbidimetric tests are applied to certain official chemicals to ensure the absence of excessive amounts of chloride and sulfate. The following directions are given in the U.S.P. for the performance of these tests:

> In carrying out these turbidimetric tests, the following points are to be observed: The same quantities of the same reagents must be used in the test of the substance under examination and in the control test. The glass cylinders in which the tests are made must be of the same diameter and match in all other respects as closely as possible. The precipitating reagent must be added to both in immediate sequence.
>
> If the solution, after the addition of acid, is not perfectly clear, it is

filtered through a filter paper free from chloride or sulfate, then the silver nitrate or the barium chloride is added.

When the tests for chloride or sulfate are to be applied to a specified volume of a solution of a substance prepared as directed in the text, and the permissible limit for these impurities corresponds to 0.2 cc. or less of 0.02*N* hydrochloric or sulfuric acid, the solution is not to be further diluted. The control test is also made with the same volume of water (or other specified solvent) as in the test.

In applying the turbidimetric tests to salts of heavy metals, which normally show an acid reaction, their aqueous solutions prepared for the test are not to be neutralized.

Bismuth salts are first dissolved in a few cubic centimeters of water and 2 cc. of nitric acid, and then treated with the precipitant.

Chloride. The prescribed quantity of the substance to be tested is dissolved in from 30 to 40 cc. of water, and the solution neutralized, if necessary, with nitric acid, using litmus paper as the indicator. One cubic centimeter of nitric acid and 1 cc. of silver nitrate T.S. are added and then sufficient water to make 50 cc. After mixing well and allowing to stand five minutes protected from direct sunlight, the turbidity, if any, is compared with that produced in a control test made with the specified volume of 0.02*N* hydrochloric acid.

Sulfate. The specified quantity of the substance to be tested is dissolved in from 30 to 40 cc. of distilled water, and the solution neutralized, if necessary, with hydrochloric acid, using litmus paper as the indicator. One cubic centimeter of diluted hydrochloric acid and 2 cc. of barium chloride T.S. are added and then sufficient water to make 50 cc. After mixing well, it is allowed to stand for 10 min. and the turbidity, if any, is compared with that produced in a control test made with the specified volume of 0.02*N* sulfuric acid.

Exercise 125

Object. Limit Test for Chloride and Sulfate in Calcium Gluconate.

Materials Required. 3 Gm. of calcium gluconate.
0.02*N* hydrochloric acid.
0.02*N* sulfuric acid.
Silver nitrate T.S. (0.1*N* silver nitrate).

Barium chloride T.S. (12 Gm. of barium chloride in sufficient water to make 0 cc.)

Matched glass cylinders.

Procedure. Proceed as directed above using 1 Gm. of calcium gluconate d 1 cc. of $0.02N$ HCl in the test for chloride and 2 Gm. of calcium gluconate d 1 cc. of $0.02N$ H_2SO_4 in the test for sulfate.

Questions and Problems

1. Calculate the per cents of chloride and of sulfate permitted in calcium luconate in the official test for purity.

2. List several official substances that are required to show no turbidity when sted for chlorides and sulfates.

3. How could the volatile oil content of a highly colored solution be deter-ined nephelometrically?

4. The presence of sugar in a solution containing volatile oils alters the par-icle size when the oil is thrown out of solution. How could the volatile-oil ontent of a cordial be determined by means of the nephelometric method?

5. Acetone is precipitated from solutions containing as little as 1 part in 00,000,000 by silver-mercury cyanide solution. Could the nephelometric ethod be used to estimate the presence of small amounts of acetone in alcohol, n ether, and in urine?

6. How might minute quantities of each of the following ions be determined: odide, sulfate, phosphate, and calcium?

7. Could minute quantities of alkaloids in solution be estimated nephelo-etrically? Suggest possible precipitants.

Those who desire to make a more extended study of optical methods of analysis will find valuable information in the following book references:

1. The Association of Vitamin Chemists, Inc., "Methods of Vitamin Assay," Interscience Publishers, Inc., New York, 1951.
2. Gibb, T. R. P., "Optical Methods of Chemical Analysis," McGraw-Hill Book Company, Inc., New York, 1942.
3. Mellon, M. G., "Analytical Absorption Spectroscopy," John Wiley & Sons, Inc., New York, 1950.
4. Sandell, E. B., "Colorimetric Determination of Traces of Metals," Interscience Publishers, Inc., New York, 1950.
5. Snell, F. D., and C. T. Snell, "Colorimetric Methods of Analysis," 3d ed., D. Van Nostrand Company, Inc., New York, 1948.

Chapter 24

REFRACTOMETRIC MEASUREMENTS

The index of refraction is a physical constant frequently made use of in the determination of the identity and purity of drug and food products. In many cases, it may be used to determine quantitatively the strength and purity of solutions or the proportions in which liquids are mixed; *e.g.*, the percentage of sugar in syrup can be estimated directly from the refractivity of the solution, and the percentage of alcohol in water can also be determined in this way. Although the refractive index is a constant characteristic of many substances, such as fats, fatty oils, waxes, sugars, organic solvents, etc., it is applied almost exclusively in the official standards as a criterion of the purity of volatile oils.

Index of Refraction. When a ray of monochromatic light passes from one transparent substance into another of different optical density, it is deflected or refracted, except when it enters perpendicularly to the surface of contact between the substances. The extent and direction of the deflection are dependent upon the difference between the densities of the two substances. The angle between the ray in the first medium and a perpendicular to the dividing surface is termed the *angle of incidence* i, and the corresponding angle in the second medium is called the *angle of refraction* r. The sin of i and the sin of r are directly proportional to the velocities of light in the two mediums. The ratio $\sin i/\sin r$ is termed the *index of refraction.* Thus, the velocities of light in air and in water are in the ratio of about 4 to 3. The index of refraction of water with respect to air is therefore about 1.333. In Fig. 49, I is the less dense medium and II the more dense medium. A ray of light passing obliquely from I to II will be deflected so that the angle of refraction r will be less than the angle of incidence i, and according to the law of refraction, the relationship between these two angles ill be sin i / sin r = N/n, where n is the index of refraction of the ess dense and N the index of refraction of the more dense medium. When the angle i is increased, the angle r also is increased and attains its maximum value when the angle of incidence enters horizontally at 90 deg., termed *grazing incidence*, or at a right angle. In the latter case, since the sin of 90 deg. = 1, the above equation becomes 1 / sin r = N/n, or sin r = n/N. The angle 90 deg. is known as the *critical angle* and the ray of incident light passing from one medium to another at this angle is known as the *critical ray*. If the angle r is increased to a value at which i becomes equal to 90 deg., the beam of light will no longer pass from the first medium to the second but will travel through the first medium to the dividing surface at an angle of 90 deg. with the perpendicular to the surface. If r is smaller than this particular value, however, the ray of light will pass on through the second medium, and, if greater, all light will be reflected from the surface back into the first medium. The critical ray is thus seen to make a boundary between the reflected and refracted light. This furnishes the basis for the observation of the refractive end point of a substance, and it is therefore used in many refractometers.

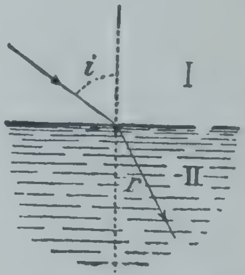

FIG. 49. Refraction of light.

Refractometers. Indices of refraction may be determined by means of a polarizing microscope or refractometer. Refractometers are of several types. Some of these give figures on an arbitrary scale, the refractive index being found by reference to a table furnished with the instrument. The principal instruments in use are (1) the Pulfrich refractometer, an instrument adapted for use with liquids and solids of all degrees of refractive power, used chiefly for purely scientific investigations; (2) the Zeiss butyro-refractometer, designed especially for the examination of fixed oils and fats; (3) the Zeiss immersion refractometer, especially constructed for the examination of aqueous, oily, or alcoholic liquids, when a large amount of sample is available; (4) the Abbé refractometer, an instrument designed for the examination of liquids, solids, and plastic bodies. Since this last instrument is best suited for the examination of all the official substances for which refractive indices are given, it is described in considerable detail.

The Abbé Refractometer. Because of its simplicity, ease of manipulation, and the small amount of material required in its use, the Abbé refractometer is extensively used in pharmaceutical analysis. The wide range of indices measurable with this instrument and the fact that it can be used with ordinary white light make it capable of more general application than any of the other refractometers mentioned above. When measuring the refractive index of liquids, only a few drops, sufficient to form a film, need be placed between the prisms of the instrument. This is a distinct advantage over the other forms of refractometers, for frequently only a small quantity of the substance to be examined is available, and if the liquid is colored or turbid, the thinness of the film permits a determination of the index of refraction without difficulty.

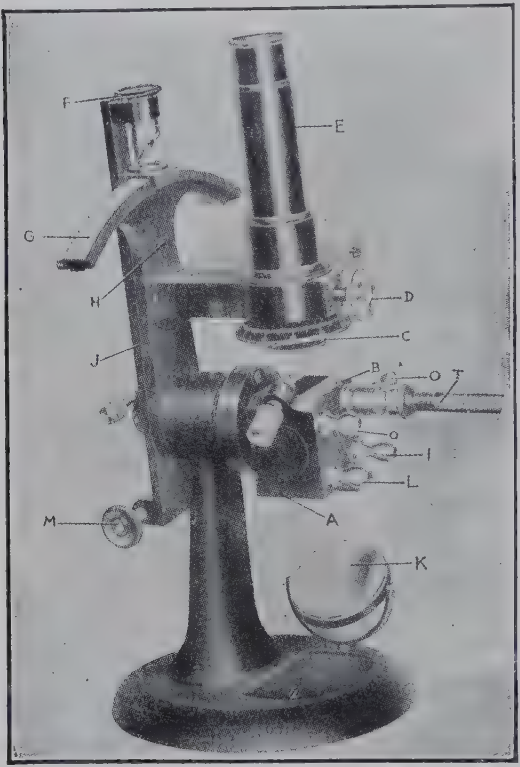

Fig. 50. Abbé refractometer.

The Abbé refractometer (Figs. 50 and 51) consists of four essential parts, *viz:* (1) a telescope *E* provided with an objective, an eyepiece, and a cross-hair disk. The purpose of the telescope is to form an image of the border line of total reflection at the intersection of the cross hairs, where the image may be observed. (2) Two similar glass prisms (Abbé prisms) of high refractive index, cemented into hollow, water-jacketed, mounting cases, so constructed that, when temperature control is required, water can be circulated about the prisms. The exposed surfaces of the upper prism *B* are highly polished, and the hypotenuse surface of the lower prism *A*, which serves solely for the purpose of illumination, is finely ground. The lower prism mount is hinged to the upper prism mount, and the latter is rigidly attached to the index arm or alidade. When the two prisms are clamped together, a space of from 0.1 to 0.15 mm. separates their surfaces. (3) The sector *H* is a metal arm to which the telescope is rigidly attached. One end of the sector is attached to the upright of the base in such a manner that the whole sector can be rotated about an axis, which coincides with the axis of rotation of the Abbé prism, so that various conditions of illumination can be accommodated. On the upper end of the sector a scale *G* is mounted, which is graduated directly in terms of the refractive index of the *D* line (sodium light) at 20°. An alidade or movable arm *J* rests against the sector, but it can be rotated as a unit with the Abbé prism independently of the sector. The upper end of the alidade bears an index mark, which moves along the edge of the sector scale when the arm is rotated. A magnifier *F* is attached to the alidade in most instruments to facilitate the reading of the index scale. A screw *M* is used to make fine adjustments of the border line at the cross hairs. (4) Compensating prisms are used to correct for the dispersion of light. Thus, when white light is used in the place of sodium light, the beam is split up into colored beams, since short wavelengths of light are refracted to a greater extent than are long wavelengths. Unless this dispersion is compensated for in some way, the border line becomes a broad, varicolored band, which cannot be brought into accurate

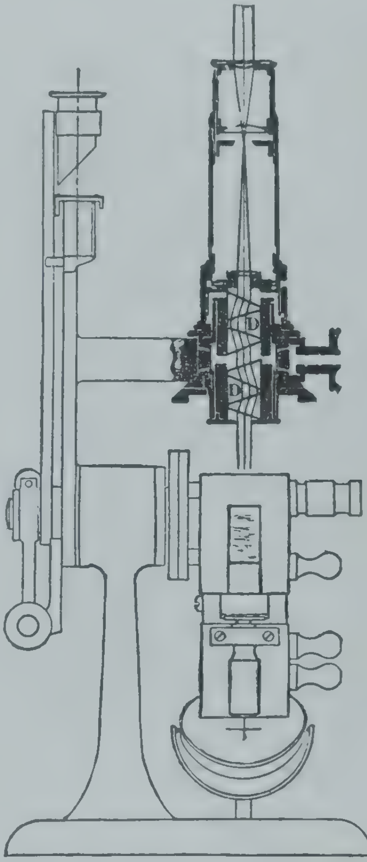

FIG. 51. Path of light, etc., through Abbé refractometer.

coincidence with the cross hairs of the telescope. The compensator functions to bring the various wavelengths of light together again. It is placed between the upper prism and the eyepiece and is rotated by means of the milled head *D*.

Regulation of Temperature. Since the density of both the prisms and the substance under investigation varies with the temperature, it is necessary in refractivity measurements of any degree of accuracy to be able to maintain the prism and the substance at constant temperature. In the Abbé refractometer, this control of temperature is accomplished by circulating water from a thermostat or other suitable constant-temperature device about the jacketed prisms, the water entering the inlets *I* and *L* passing out of the outlets *O*, the temperature at which the measurement is made being observed on the thermometer *T*. A temperature control device is illustrated in Fig. 52.

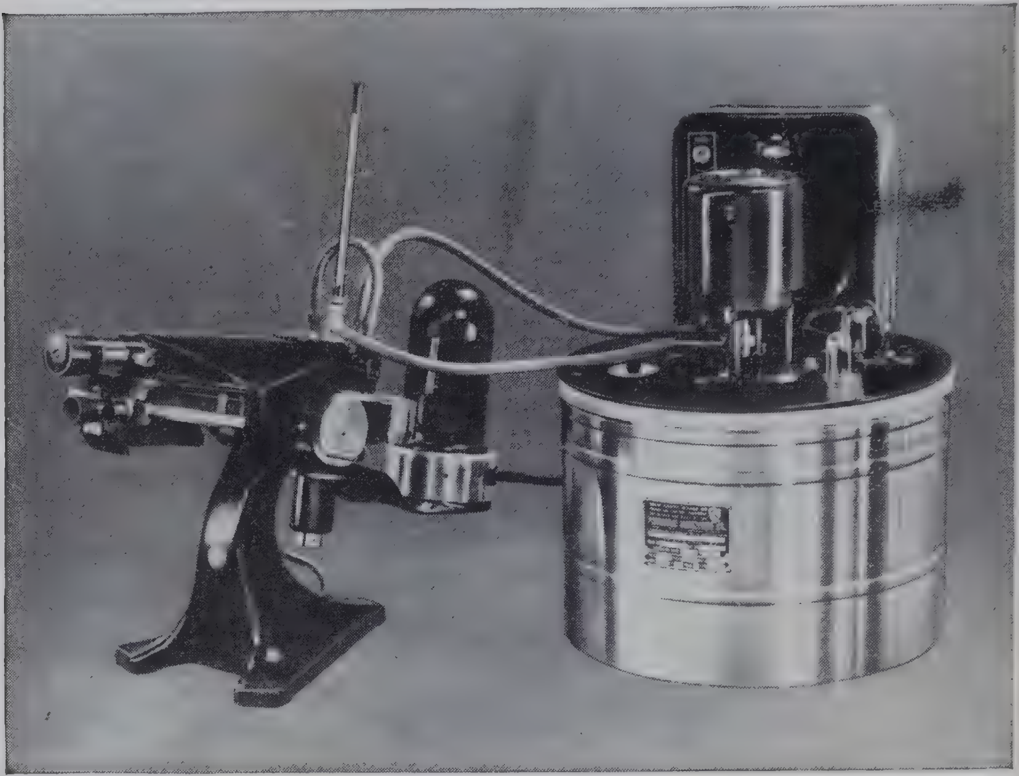

FIG. 52. Precision refractometer and precision temperature controller. (*Courtesy of Bausch & Lomb Optical Co.*)

Exercise 126

Object. To Determine the Refractive Index of Orange Oil.

Materials Required. About 1 cc. of orange oil.
A pipette.
An Abbé refractometer.

Procedure. 1. Rotate the prisms and telescope so that the lower prism may be swung open easily. Attach the test plate to the upper prism, by means of the monobrom-naphthalene or other liquid provided with the test plate, by moistening the test plate with the liquid and pressing it against the upper prism *B*. Focus the light on the test plate, rotate the prism by means of the alidade until the border line of total reflection coincides with the cross hairs in the telescope, and take the reading of the refractive index.

The refractive index of the test plate is etched upon it, and the reading of the instrument should correspond to that given on this plate. If the instrument is not in adjustment, a correction should be applied to the readings obtained, or adjustment should be made.

2. Remove the test plate, clean the upper prism with cotton saturated with alcohol, clamp the prisms together loosely, introduce 2 or 3 drops of the volatile oil into the groove at the side of the prisms, and clamp the prisms together firmly. Adjust the mirror *K* so that the light is reflected upon the lower prism, and rotate the alidade until the border line between the light and dark halves of the field of view exactly coincides with the cross hairs of the telescope, rotating the compensator prisms to obtain a sharp uncolored border line if necessary. Read the refractive index of the oil directly from the graduated sector scale to the fourth decimal place. Move the alidade and again determine the refractive index until three readings are obtained, taking the mean of the readings as the refractive index of the oil. Note the temperature at which the refractive index is read.

The readings on the graduated scale of the compensator drum may be referred to tables of mean dispersion and conversion supplied with the instrument. The refractive index of the oil should be expressed as, *e.g.*, $N_D^{20^\circ} = 1.4725$ where $N_D^{20^\circ}$ denotes the index of refraction for the *D* line (sodium light) measured at 20°.

Questions and Problems

1. Define the terms *index of refraction*, *critical ray*, and *angle of incidence*.

2. What advantages does the Abbé refractometer possess over the other types of refractometers?

3. A range is given for the refractive index of most of the official volatile oils. Why?

4. How do variations in temperature affect the indices of refraction of liquids?

5. Would you expect that orange oil would have a greater or smaller index of refraction at 25 than at 20°? Why?

TABLE 73. OFFICIAL SUBSTANCES WITH REQUIRED REFRACTIVE INDICES

Substance	Official requirement*	Substance	Official requirement*
U.S.P.		**U.S.P.**	
Anise oil	1.5530 to 1.5600	Sassafras oil	1.5250 to 1.5350
Benzyl benzoate	1.5680 to 1.5700	Spearmint oil	1.4840 to 1.4910
Butopyronoxyl	1.4745 to 1.4755	Theobroma oil	1.4537 to 1.4585‡
Cherry juice	(n.l.t.) 1.3500	Triethanolamine	1.481 to 1.486
Cinnamon oil	1.6202 to 1.6135	**N.F.**	
Clove oil	1.5300 to 1.5350	Almond oil, bitter	1.5410 to 1.5442
Coriander oil	1.4620 to 1.4720	Anethole	1.5570 to 1.5610§
Dimethyl phthalate	1.5130 to 1.5170	Benzaldehyde	1.5440 to 1.5465
Ethohexadiol	1.4465 to 1.4515	Benzyl alcohol	1.5385 to 1.5405
Eucalyptol	1.4550 to 1.4600	Bergamot oil	1.4650 to 1.4675
Eugenol	1.5400 to 1.5420	Caraway oil	1.4840 to 1.4880
Fennel oil	1.5280 to 1.5380	Cardamon oil	1.4630 to 1.4660
Glyceryl triacetate	1.4288 to 1.4296	Chenopodium oil	1.4740 to 1.4790
Lavender oil	1.4590 to 1.4700	Cinnamaldehyde	1.6180 to 1.6230
Lemon oil	1.4740 to 1.4755	Coconut oil	1.4477 to 1.4495‡
Methyl salicylate	1.5350 to 1.5380	Eucalyptus oil	1.4580 to 1.4700
Myristica oil (East		Isopropyl alcohol	1.3770 to 1.3780
Indian)	1.4740 to 1.4880	Juniper oil	1.4740 to 1.4840
Myristica oil (West		Myrcia oil	1.5070 to 1.5160
Indian)	1.4690 to 1.4760	Orange, bitter, oil	1.4725 to 1.4755
Nikethamide	1.522 to 1.524	Pimenta oil	1.5270 to 1.5400
Orange oil	1.4723 to 1.4737	Pine needle oil, dwarf	1.4750 to 1.4800
Peanut oil	1.4625 to 1.4645	Pine oil	1.4810 to 1.4840
Peppermint oil	1.4590 to 1.4650	Santal oil	1.5000 to 1.5100
Raspberry juice	(n.l.t.) 1.3445	Thyme oil	1.4950 to 1.5050
Rose oil	1.4570 to 1.4630†	Turpentine oil	1.4680 to 1.4780
Rosemary oil	1.4640 to 1.4760	Undecylenic acid	1.4475 to 1.4485

* Refractive index at 20° unless a different temperature is specified.

† 30°.

‡ 40°.

§ 25°.

(n.l.t.) = not less than.

Chapter 25

ROTATORY POWER

Many organic substances, such as certain constituents of the volatile oils, alkaloids, and sugars, possess the power of rotating the plane of polarized light when the latter is passed through solutions containing them. Substances that possess this power are said to be *optically active* and are designated as dextrorotatory when the direction of the rotation is toward the right and as levorotatory when it is toward the left.

The optical activity of many substances is a function of their chemical constitution as well as of their concentration. Consequently, a determination of the rotatory power or lack of rotatory power of a substance may serve as a means of establishing both its identity and its purity. In some cases, the measurement of the optical activity of a substance may also give some indication of its therapeutic value; *e.g.*, the levorotatory alkaloid hyoscyamine is much more active as a mydriatic than its optically inactive isomer atropine.

In a ray of *ordinary light*, the vibrations are transverse, *i.e.*, they take place in a plane at right angles to the direction of propagation, but the vibration direction is constantly changing. In a ray of *plane-polarized light*, commonly termed *polarized light*, the vibrations are also transverse, but they take place in only one direction.

The polariscope and the polarizing microscope are used in the determination of the optical activities of liquid and solid substances, the polarizing microscope being employed more often for the examination of solid substances, while the polariscope is used primarily for the examination of liquids.

When a ray of ordinary light is passed through a specially constructed prism made of Iceland spar, such as the Nicol prism, the emergent light wave vibrations take place in one plane, and the light is said to be *plane-polarized*. If another Nicol prism is used to examine the plane-polarized light, it will be found that, on rotating the latter prism, the field of view appears alternately light and dark, the minimum brightness following the maximum as the prism is rotated through an angle of 90 deg. The prism by means of which the light is polarized is termed the *polarizer*, and the second prism, by means of which the light is examined, is called the *analyzer*.

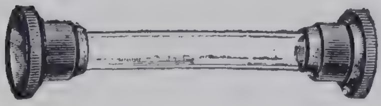

FIG. 53. Polariscope tube.

If, when the field of view appears dark, as when the axes of the two prisms are at right angles to each other, a tube similar to that shown in Fig. 53 or 54 is filled with an optically active liquid and placed between the polarizer and the analyzer, the field will become light because of the rotation of the plane of polarization by the optically active substance. The extent to which the plane of polarization has been rotated can be determined by turning the analyzer through a certain angle until the field becomes dark again. When it is necessary to turn the analyzer to the right (clockwise), the optically active substance is said to be *dextrorotatory;* when the analyzer must be turned to the left (counterclockwise), it is said to be *levorotatory*.

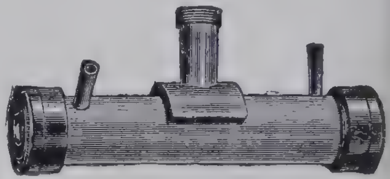

FIG. 54. Polariscope tube with jacket and thermometer well for constant temperature control.

It is possible to obtain a position at which the field of view becomes dark by rotation of the analyzer to either the right or left, since in one complete rotation of the prism through 360 deg., there are two positions of the analyzer, 180 deg. apart, at which the field becomes dark, and, likewise, two positions at which there is a maximum of light. In determining the sign of activity of a given substance, it is customary to take the direction in which the rotation required to reproduce the dark field is less than 90 deg.

The angle of rotation of the polarized light on passage through a substance varies directly as the length of the column of substance through which the light passes. In homogeneous mixtures or solutions, the angle of rotation depends upon the concentration of the optically active substances present. When more than one optically active substance is present in a solution, the resultant rotation will be the same as the sum of the several rotations of each separate constituent in the same volume of solution, provided that the substances do not react with each other. The angle of rotation also varies with the wavelength of the light employed (the shorter the wavelength the greater the angle of rotation), with the temperature, and with the nature of the substance. When all these factors are taken into account, the results may be calculated in terms of specific rotation or specific rotatory power.

The specific rotation $[\alpha]$, of a liquid is defined as *the angular rotation in degrees through which the plane of polarization of polarized monochromatic light is rotated by passage through* 1 *decimeter* (100 *mm.*) *of the liquid, calculated to the basis of a specific gravity of* 1. *In the case of solutions of an optically active substance, the specific rotation is calculated to the basis of a concentration of* 1 *Gm. of solute in* 1 *cc. of solution.*

Since the specific gravity of liquids, as well as the optical activity of many substances, is influenced by the temperature, it is necessary to indicate the temperature at which the rotation and the specific gravity are determined. The temperature specified in the two official standards for the measurement of optical activity is generally 25°, except for sugar, which is determined at 20° by international agreement.

The calculation of the specific rotatory power of an optically active liquid or solution of an optically active solid may be made from the following general formulas:

1. For liquid substances $[\alpha]_D^t = \dfrac{a}{ld}$

2. For solutions $[\alpha]_D^t = \dfrac{100a}{lpd}$

$$\text{or } [\alpha]_D^t = \frac{100a}{lc}$$

For calculating the specific rotation $[\alpha]$ using these formulas, the determination of the following factors is necessary:

a = observed rotation in degrees of the liquid at temperature t using a sodium light.

l = the length of the tube in decimeters.
d = the specific gravity of the liquid or solution at the temperature of observation.
p = concentration of solution expressed as the number of grams of active substance in 100 Gm. of solution.
c = concentration of solution expressed as the number of grams of active substance in 100 cc. of solution.

In expressing the specific rotation of a substance in solution, the concentration and solvent used must always be stated. The temperature should also be recorded; *e.g.*, $[\alpha]_D^{20°} = -38°$ means that the specific rotation of the substance determined at 20° using sodium light is levorotatory 38 deg., and $[\alpha]_D^{20°} = +16°$ means that the substance is dextrorotatory 16 deg. when the specific rotation is determined at 20°, using sodium light.

Polarimeters. The instruments used to measure the optical activity of liquid substances are called *polariscopes* or *polarimeters* and *saccharimeters*. Polarimeters permit the angle of rotation to be read off in degrees or fractions of a degree of a circle, and saccharimeters are usually constructed and set to give readings directly in terms of percentage of sugar. The so-called "half-shadow" instruments known as the *Laurent* and the *Schmidt and Hänsch* polarimeters are the most generally used in pharmaceutical work.

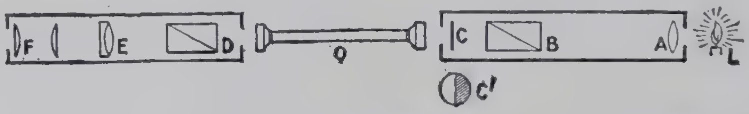

FIG. 55. Diagram of the optical parts of a polarimeter.

In the polarimeter of Laurent and that of Schmidt and Hänsch, the optical parts may be represented diagrammatically as in Fig. 55. Monochromatic light (sodium light) from the source *L* passes through the lens *A*, which renders the rays of light parallel, and then passes through the polarizing prism *B*, which renders the light ray plane-polarized. It then passes through the observation tube *O*, which contains the substance being examined, and thence through the analyzer prism *D*. The field of view is observed through the telescope *FE*. The circular opening of the tube carrying the polarizer is one-half covered by a thin quartz plate as illustrated in *C′*, the thickness of which is so adjusted that the light in passing through the plate is altered in phase by half a wavelength but remains plane-polarized. In this way, two beams of polarized light are produced. If the polarizer is rotated so that the plane of polarization forms an angle X with the quartz plate, the planes of polarization will also be inclined at an angle $2X$. This is termed the *half-shadow angle.* On rotating the analyzer, a position will be found at which one beam of light will be completely, and the other only partly, extinguished. One half of the field of view will then appear dark while the other half will remain light. Upon rotating the analyzer farther through the angle $2X$, a second position will be found at which the second beam of light will be extinguished while the first beam becomes visible. At this position of the analyzer, the half of the field which formerly appeared dark will be light, and that which was formerly light will be dark. When the analyzer occupies an intermediate position, a field of view of uniform brightness will appear, and this is the position at which the analyzer should be set.

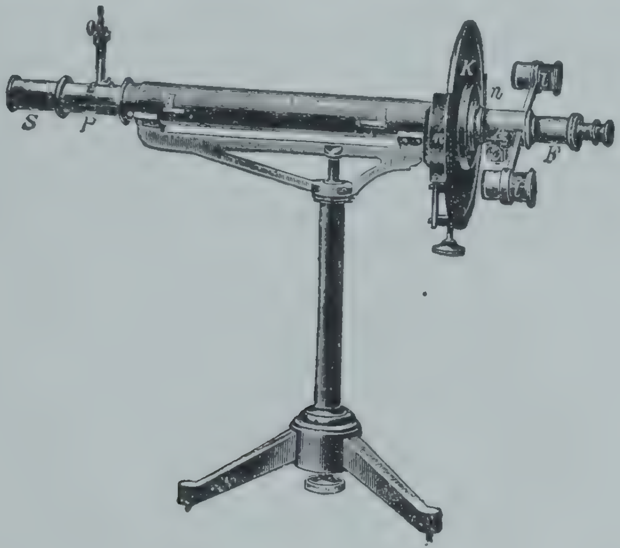

FIG. 56. A polarimeter.

The complete polarimeter is shown in Fig. 56. At the end S, which is directed toward the source of illumination, is the lens. The polarizing prism is at P, and it is connected with a lever L, by means of which it can be rotated to alter the angle of half-shadow. The observation tube is placed in the central part of the instrument an protected from extraneous light by a hinged cover. The analyze is placed at A, and it can be rotated, independently of the graduate circle, by means of a screw. This permits the correction of the zer point of the instrument. F is a telescope with an eyepiece by mean of which the field of view is observed. K is a graduated disk, whic can be made to rotate with the analyzer and telescope past the fixe verniers n on the graduated circle. Most instruments are provide with one or more magnifying lenses to increase the accuracy o readings.

The official polarimetric determinations should be carried out i a dark room using monochromatic light. Monochromatic light can be obtained easily by introducing a fused bead of sodium chloride on a platinum wire or gauze into the nonluminous flame of a gas burner.

The observation tube, in which the liquid to be examined is placed, usually consists of a tube of thick glass with accurately ground ends, closed by circular glass plates with parallel sides, which are pressed against the ends of the tube by means of screw caps. Other polarimeter tubes are constructed with a jacket through which water may be circulated to give a constant temperature. Since the unit of length in polarimetric measurements is 1 dcm., the observation tubes are generally of 1-dcm. length or some multiple or fraction thereof, *e.g.*, 2.0, 0.5, and 0.25 dcm. The length of the tube used in any definite determination usually depends upon the depth of color in the liquid and the magnitude of its rotatory power.

Readings made on a saccharimeter may be converted into the angular degrees of a polarimeter by means of the following factors:

1 division of the Laurent sugar scale = 0.2167 deg. angular rotation D.

1 division of the Ventzke or Schmidt and Hänsch sugar scale = 0.3468 deg. angular rotation D.

Exercise 127

Object. To Determine the Specific Rotation of Sucrose.

Materials Required. 26 Gm. of cane sugar.
A 100-cc. volumetric flask calibrated at 20°.

A polarimeter with a 200-mm. observation tube.

Procedure. 1. Adjust and determine the zero point of the polarimeter.

The polarimeter should be set up so that the polarizer end is in direct line with the bright sodium flame and about 4 to 6 in. from it. Place a 200-mm. observation tube filled with distilled water in the tube holder between the polarizer and the analyzer and focus the telescope eyepiece on the line bisecting the field of view, rotating the analyzer if necessary in order to obtain unequal illumination of the two halves of the field. Now determine the zero point by rotating the analyzer until equal illumination of the two halves of the field is obtained. This position should be approached several times from either side of the zero point, readings being made from the verniers, and the mean of at least four readings taken as the zero point. Since the zero point is altered by the alteration of the angle of half-shadow, the lever that rotates the polarizer must be set before the zero point is determined. It is possible to rotate the analyzer in some instruments without rotating the graduated disk, and it is possible, in such cases, to adjust the instrument so that the zero-point correction is eliminated.

2. Dissolve 26 Gm. of sucrose, accurately weighed and previously dried to constant weight at 105° in sufficient distilled water to make 100 cc. at 20°.

The sugar is dried to drive off moisture. The solution should be made up in a volumetric flask calibrated at 20°, and the final adjustment of the volume should be made at the same temperature.

3. Fill a clean dry observation tube with the solution. Determine the value of the angle of rotation. Calculate the specific rotation of the sample examined.

A polarimeter tube may be cleaned and dried easily by washing it successively with water, alcohol, and ether. In the determination of the angle of rotation, a number of successive readings should be taken as described under Procedure 1, and their mean taken as the angle of rotation. After correcting for the zero point of the instrument, the specific rotation can be calculated by the formulas given on page 489.

The specific rotation of the official sucrose must not be less than +65.9 deg. when determined by the above method. Compare the specific rotation of the sample examined with the official requirement.

TABLE 74. OFFICIAL SUBSTANCES WITH REQUIRED SPECIFIC ROTATION

Substance	Solvent used	Concentration in 100 cc., Gm.	Specific rotation*
U.S.P.			
Apomorphine hydrochloride	0.02N HCl	1.5	−49 to −51
Ascorbic acid	Water	10	+20.5 to +21.5
Calciferol	Acetone	1.6	+79.5 to +83.5
Camphor	Alcohol	10	+41 to +43
Chloramphenicol	Dehydrated alcohol	5	+17 to +20
Cholesterol	Dioxan	2	−34 to −38
Cocaine hydrochloride	Water	2	−71 to −73
Codeine sulfate	Water	2	−112.5 to −115
Colchicine	Water	1	−410 to −435
7-Dehydrocholesterol, activated	Alcohol	0.32	+103 to +112
Desoxycorticosterone acetate	Dioxan	1	+168 to +176
Dextrose	Water†	10	+52.5 to +53
Digoxin	Anhydrous pyridine	10	+13.5 to +14.1‡
Dihydromorphinone hydrochloride	Water	2	−136 to −139
Ephedrine	Water	5§	−33 to −35.5
Ephedrine hydrochloride	Water	5	−33 to −35.5
Ephedrine sulfate	Water	5	−29.5 to −32.0
Epinephrine	0.5N HCl	2	−50 to −53.5
Ergonovine maleate	Water	1	+48 to +57
Ergotamine tartrate	Chloroform	0.34¶	(n.l.t.) −150[a]
Estradiol	Dioxan	1	+76 to +83
Estradiol benzoate	Dioxan	1	+58 to +63
Estrone	Dioxan	1	+155 to +166
Ethisterone	Pyridine	1	+28 to +33
Ethylmorphine hydrochloride	Water	2	−102 to −105
Lactose	Water†	10	+52.2 to +52.5
Lanatoside C	Alcohol	2	+33.4 to +33.7[a]
Menthol (dl-)	Alcohol	10	−2 to +2
Menthol (l-)	Alcohol	10	−45 to −51
Methamphetamine hydrochloride	Water	2	+16 to +21
Methyltestosterone	Benzene	1	+84 to +87
Morphine sulfate (anhydrous)	Water	2	−107 to −109.5
Ouabain	Water	1	−31 to −32.5
Physostigmine salicylate	Water	1	−91 to −94
Pilocarpine hydrochloride	Water	2	+87.5 to +90
Pilocarpine nitrate	Water	2	+78 to +80
Progesterone	Dioxan	2	+173 to +183[b]
Riboflavine	0.1N NaOH in dilute alcohol	0.5	−112 to −122
Scopolamine hydrobromide	Water	5	−24 to −26
Sucrose	Water	26	(n.l.t.) +65.9
Testosterone	Dioxan	1	+101 to +105
Testosterone pellets	Dioxan	1	+101 to +105
Testosterone propionate	Dioxan	1	+83 to +90
Tubocurarine chloride	Water	1	+208 to +218[b]
N.F.			
Chaulmoogra oil	Chloroform	10	+48 to +60
Ephedrine sulfate solution	Water	[c]	−29 to −32
Glutamic acid hydrochloride	3N HCl	12.5	+24 to +26
Histidine monohydrochloride	1N HCl	2.4	+9.7 to +11.2
Salicin	Water	3	−62 to −67
Santonin	Alcohol	2	−170 to −175

---

* Specific rotation at 25° using D-line of sodium unless otherwise specified.
† Add 0.2 cc. of ammonia T.S.
‡ Using Hg light at 546.1 mμ.
§ Hydrochloride prepared from ephedrine.
¶ Ergotamine prepared from 0.34 Gm. of ergotamine tartrate.
[a] At 20°.
[b] After standing for 3 hr.
[c] Calculated from analysis.
(n.l.t.) = not less than.

TABLE 75. OFFICIAL SUBSTANCES WITH REQUIRED ANGULAR ROTATION

Substance	Official requirement* angular rotation, deg.
U.S.P.	
Acacia	Slightly levorotatory†
Anise oil	−2 to +1
Cinnamon oil	−1 to +1
Clove oil	(n.m.t.) −1.5
Coriander oil	+8 to +15
Eucalyptol	+0.3 to −0.3
Fennel oil	+12 to +24
Lavender oil	−3 to −10
Lemon oil	+57 to +65.6
Methyl salicylate (synthetic and from betula)	Inactive
Methyl salicylate (from gaultheria)	(n.m.t.) −1.5
Myristica oil (East Indian)	+10 to +30
Myristica oil (West Indian)	+25 to +45
Orange oil	+94 to +99
Peppermint oil	−18 to −32
Rose oil	−1 to −4
Rosemary oil	−5 to +10
Sassafras oil	+2 to +4
Spearmint oil	−48 to −59
Tubocurarine chloride injection	+0.35 to +0.40‡
N.F.	
Almond oil, bitter	(n.m.t.) +0.167
Anethole	(n.m.t.) 0.15
Bergamot oil	+8 to +24
Caraway oil	+70 to +80
Cardamon oil	+22 to +44
Chenopodium oil	−4 to −8
Cinchonidine sulfate	Levorotatory
Cinchonine sulfate	Dextrorotatory
Juniper oil	0 to −15
Myrcia oil	(n.m.t.) −3
Orange, bitter, oil	+88 to +98
Orange flower oil	+1.5 to +9.1
Pimenta oil	0 to −4
Pine needle oil, dwarf	−5 to −15.5
Quinine	Levorotatory§
Santal oil	−15 to −20
Strychnine nitrate	Levorotatory
Thyme oil	(n.m.t.) −3
Turpentine oil	Variable

* In a 100-mm. tube.

† 10 per cent solution.

‡ Rotation for each milligram per cubic centimeter claimed on label. Determined in a 200-mm. tube.

§ Alcohol solution, 1 to 10.

(n.m.t.) = not more than.

Questions and Problems

1. What is polarized light? How can it be obtained?

2. Show diagrammatically the essential parts of the polariscope used and explain the function of each part.

3. What factors influence the rotatory power of a substance?

4. Define the term *specific rotatory power.*

5. Explain what the following terms mean: (1) $[\alpha]_D^{25°} = +1.210$; (2) $[\alpha]_D^{25°} = -10°$; (3) *dextrorotatory;* (4) *optically inactive.*

6. The optical rotation of a sample of oil of chenopodium determined in a 100-mm. tube and having a density of 0.9750 is found to be −12.25 deg. at 21°. Calculate the specific rotation of the oil.

7. A solution containing 10 Gm. of sugar in 100 cc. was found to exhibit an angular rotation of +11.5 deg. when determined in a 100-mm. observation tube. Calculate the specific rotation of the sucrose.

The measurement of rotatory power is used as a means of assaying for dextrose in dextrose and sodium chloride injection and dextrose injection in the U.S.P. The official U.S.P. method for assaying for methyltestosterone in methyltestosterone tablets also depends on the determination of optical rotation.

Those substances for which the angular rotation at 25° is determined in a 100-mm. observation tube are listed in Table 75. Those which have specific rotation requirements are listed in Table 74.

Chapter 26

SOLUBILITY

The statements of the solubilities of official substances are given primarily for the information of physicians and pharmacists for use in the preparation and dispensing of medicines. They should not always be regarded as exact physical constants, upon which the identification or the determination of the purity of a substance may be based. Frequently, the official standards are descriptive terms to indicate the approximate solubility of substances, *viz.:* (1) "Very soluble," 1 part of the substance is soluble in less than 1 part of the solvent; (2) "freely soluble," 1 part of the substance is soluble in from 1 to 10 parts of solvent; (3) "soluble," 1 part of the substance is soluble in from 10 to 30 parts of solvent; (4) "sparingly soluble," 1 part of the substance is soluble in from 30 to 100 parts of solvent; (5) "slightly soluble," 1 part of the substance is soluble in from 100 to 1,000 parts of solvent; (6) "very slightly soluble," 1 part of the substance is soluble in from 1,000 to 10,000 parts of solvent; (7) "practically insoluble," more than 10,000 parts of solvent are required to dissolve 1 part of the substance.

There are nine possible classes of solutions, *viz.:* (1) a gas in a gas; (2) a gas in a liquid; (3) a gas in a solid; (4) a liquid in a gas; (5) a liquid in a liquid; (6) a liquid in a solid; (7) a solid in a gas; (8) a solid in a liquid; (9) a solid in a solid. Although examples of all these different types of solutions are known, the only classes of pharmaceutical importance are solutions of gases in liquids, of liquids in liquids, and of solids in liquids.

The official determination of the solubility of gases in water, such as HCl and NH_3, is generally effected by chemical titration methods, while the solubility of liquids in liquids and of solids in liquids may be determined by physical methods, such as specific gravity, optical rotation, refractive index, etc., as well as by usual titrimetric or gravimetric analytical methods.

Numerous factors affect the rate and the extent of the solubility of a substance in a given solvent, as follows:

1. The solubility of most of the official substances is increased by a rise in the temperature at which solution is effected. There are, however, numerous exceptions to this generality; *e.g.*, gases, calcium salts, and ether are less soluble in hot than in cold water. Since the solubility of substances varies markedly with slight changes in temperature, it is very important that a constant temperature be accurately maintained throughout a solubility determination.

2. Substances in a fine state of division dissolve more rapidly than large crystals or particles because the total surface area exposed to the action of the solvent is much greater when the substance is powdered.

3. The purity of the substance and of the solvent must be assured in all solubility determinations, since slight amounts of impurity in either may cause considerable variation in the results. In the official statements of solubility, it is assumed that both the substance and the solvent employed conform to the official tests for purity.

4. The position of the solute in the solvent affects the rate of solution. If the solute is allowed to lie on the bottom of the vessel, it becomes surrounded by a layer of concentrated solution, which prevents the access of fresh portions of the solvent to the surface of the solute.

The determination of the solubility of a solid in a liquid necessitates the preparation of a saturated solution at a carefully controlled temperature of the substance. This is followed by ascertaining, by some type of analysis, the amount of the substance present in a definite quantity of the solution. Complete saturation can be attained most readily by agitation of an excess of finely divided solid material in the appropriate solvent contained in a closed vessel. The agitation may be accomplished by means of a mechanical stirrer or by shaking. At periodic intervals the undissolved material is allowed to settle and an accurately measured sample of the solution is removed for analysis. When two consecutive results agree, saturation of the solution is known to be complete. Such determinations must be made in a temperature-controlled atmosphere, such as through the use of a constant-temperature water bath.

Exercise 128

Object. To Determine the Solubility of Boric Acid in Water at 25°.

Materials Required. 1.5 Gm. of boric acid.

A solubility apparatus and thermostat.

Procedure. 1. In a suitable container, place about 1.5 Gm. of finely powdered boric acid and 25 cc. of distilled water. After fitting it with the stirrer, place it in the thermostat so that it is immersed in the water up to the level of the stopper, and stir the solution vigorously for about 3 hr.

The boric acid is powdered finely because substances in fine particles dissolve more rapidly than do large crystals; subdivision increases the surface area exposed to the action of the solvent.

The container is immersed in the thermostat bath to maintain a constant temperature throughout the mixture, since the solubility of boric acid varies appreciably with slight changes in temperature. The temperature in the thermostat should not vary by more than 0.1°, above or below 25°, during the determination. The mixture is stirred continuously to bring fresh portions of solvent in contact with the undissolved particles of boric acid and to ensure equal concentration throughout the solution.

2. Stop the stirrer and remove it from the tube, stopper the latter securely, allow the undissolved crystals to settle, remove about 5 cc. of the clear solution from the solubility tube by means of a pipette, and weigh accurately. Evaporate this portion of the solution to dryness on a water bath at 80° and finally to constant weight at 90° in an oven, and weigh the residue.

The weight of the boric acid contained in the residue, subtracted from the weight of the solution analyzed, gives the weight of the water in which the boric acid was dissolved.

3. Attach the stirrer and allow the stirring to continue for about 1 hr. Again remove a 5-cc. portion of the solution, weigh it accurately, and determine the amount of boric acid it contains, in the same manner as before.

If this result agrees with the former determination, it shows that saturation of the solution is complete, but if the amount of dissolved solid is greater in the second case, the stirring must be continued until the concentration of the solution becomes constant.

4. Express the results as grams of boric acid soluble in 100 Gm. of water.

If a 5.5-Gm. portion of a saturated boric acid solution yielded 0.3000 Gm. of boric acid upon drying to constant weight, the 0.3 Gm. of boric acid was dissolved in $5.5 - 0.3 = 5.2$ Gm. of water. The amount of boric acid soluble in 100 Gm. of water at 25° may therefore be calculated from the simple proportion

$$0.3:5.2::X:100$$

in which X = the number of grams of boric acid soluble in 100 Gm. of water at the temperature at which the determination was carried out.

Calculate the solubility of boric acid in water at 25° from the results of the above determination.

Questions and Problems

1. What factors influence the rate of solution of boric acid in water?

2. Why is the accurate control of temperature throughout a solubility determination essential?

3. Why is it necessary to carry out a solubility determination in a closed container?

Chapter 27

VISCOSITY MEASUREMENTS

The viscosity of a fluid substance is constant for any given temperature and is a measurable characteristic of the substance. It is used chiefly as an index of the composition and lubricating value of oils. The viscosities of solutions and liquid mixtures often vary with their concentration and composition; this property, however, may be used, in many cases, as a rapid means of analysis. The U.S.P. utilizes the viscosity as a means of standardizing liquid petrolatum, which is employed therapeutically as an intestinal lubricant.

Fluidity is the reciprocal of viscosity; *i.e.*, fluidity = 1/viscosity. Fluidity may be regarded as a measure of the tendency of a liquid to flow, whereas viscosity is a measure of the resistance that a liquid exerts against the tendency to flow. Viscosity may be defined as *the force of friction that tends to retard movement in a fluid body.* The unit of absolute viscosity is defined as *the tangential force required to move a unit area of plane surface within the liquid with unit velocity relative to another parallel unit area of plane surface one unit distant from the former surface, where the unit of force is the dyne (the force necessary to produce an acceleration of 1 cm. per second per second on a mass of 1 Gm.), the unit of velocity is the centimeter per second, the unit of area is the square centimeter, and the unit of distance is the centimeter.* This unit of absolute viscosity is known as the *poise*, and it is generally expressed as dyne-second per square centimeter. The poise is a relatively large unit, and the one-hundredth part of the poise, or a *centipoise*, is commonly employed as the unit of absolute viscosity.

Instead of giving results in terms of absolute viscosity, most methods of determination give the relative viscosity; *i.e.*, the viscosity of the liquid compared with that of some standard of known viscosity, such as water under similar conditions. Since the relative viscosities obtained by determinations with different types of apparatus are not the same, the *kinematic viscosity*, which is *the ratio of the absolute viscosity in poises to the density*, has been adopted in the U.S.P. as the method of expressing viscosity. The unit of kinematic viscosity is the *stoke*, which is equivalent to 100 centistokes. As the viscosity of a liquid changes very rapidly with the temperature, decreasing about 2 per cent for each degree rise in temperature, it is important to know the exact temperature of the liquid at the time of measurement and to use an instrument provided with some means whereby the sample under examination may be maintained at constant temperature.

Apparatus. In the official determination any suitable viscosimeter may be employed, and the results obtained with these instruments may be converted into the kinematic viscosity by the use of a conversion table. The instruments are constructed to measure the time required for a certain amount of fluid to pass through a capillary tube. When the Engler instrument is used, the viscosity is expressed in terms of *Engler degrees*, obtained by dividing the time of discharge for the liquid being tested by the time of discharge of the same volume of water at 20°. In this country, the Saybolt universal viscosimeter is most commonly employed in the determination of the viscosity of lubricating oils. The results obtained with this instrument are expressed as the time in seconds required for the discharge of the liquid being tested.

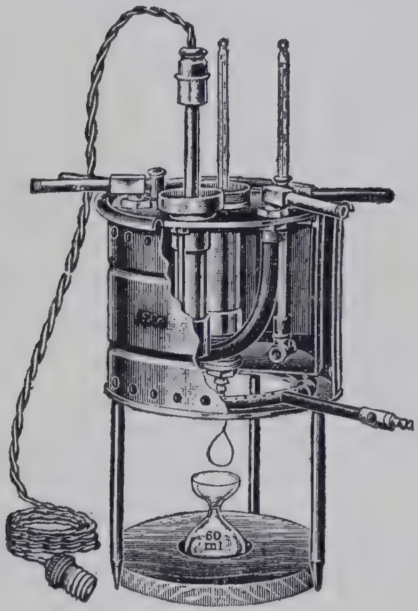

FIG. 57. Saybolt universal viscosimeter.

The *Saybolt universal viscosimeter* (Fig. 57) consists of a metallic cup of about 80-cc. capacity to contain the liquid under examination, with an outlet in the bottom closed by a cork stopper. This cup is surrounded by a jacket which serves as a constant-temperature bath. Most instruments are provided with devices for stirring and heating the bath, with a thermometer to measure the temperature, and with a receiving flask graduated to contain 60 cc. at 20°; a stop watch with which the time of discharge of the liquid may be measured is usually a part of the standard equipment supplied with the instrument, but any stop watch may be used.

Exercise 129

Object. To Determine the Kinematic Viscosity of Liquid Petrolatum.

Materials Required. A Saybolt viscosimeter.
A stop watch.
100 cc. of liquid petrolatum.

Procedure. 1. Assemble the viscosimeter, fill the constant-temperature bath with water, and bring the temperature of the latter to about 40°. Tightly stopper the outlet tube, and pour the sample of petrolatum into the oil cup until the level is above the rim of the main cup. When the temperature of the oil sample is 37.8°, pipette the excess oil from the overflow cup until the level in it is below the rim of the main cup.

The water in the temperature bath should be stirred continually to ensure an even distribution of temperature throughout the sample of oil.

2. When the thermometer in the oil cup registers exactly 37.8°, remove the cork from the outlet tube, starting the stop watch at the same instant, and collect the effluent oil in the flask graduated to hold 60 cc. When the level of the oil in the flask reaches the 60-cc. graduation mark, stop the watch.

The time in seconds required for the efflux of 60 cc. of the liquid petrolatum is called the *Saybolt universal viscosity.*

The U.S.P. requires that liquid petrolatum have a kinematic viscosity of not less than 0.381 at 37.8° (100°F.). This value is equivalent to a Saybolt universal viscosity of 177.2.

For the determination of the viscosity of methylcellulose, N.F., special liquid flow tube viscosimeters are used because solutions of the high viscosity types of methylcellulose are too viscous for use in viscosimeters commonly employed. The principle of measurement is essentially the same as that of the Saybolt viscosimeter except that larger orifice tubes are used and the design is somewhat different. The viscosimeter is standardized against an oil of known viscosity. Such oils are available from the National Bureau of Standards. The oil chosen as the standard must have a viscosity close to that of the type of methylcellulose to be determined.

The viscosity of a 2 per cent solution of methylcellulose labeled to be 15 centipoises shall not be less than 13 and not more than 18 centipoises. Higher viscosity methylcellulose samples must meet similar standards.

Questions and Problems

1. Consult the viscosity conversion table (U.S.P.) and express the results obtained in the above exercise in terms of kinematic viscosity.

2. If the viscosity of a sample of liquid petrolatum is found to be less than the official requirement, what does the determination indicate relative to the composition of the oil?

3. Would viscosity requirements be of value as a constant for (*a*) glycerin; (*b*) castor oil; (*c*) simple syrup?

4. Consult the N.F. IX, and explain the formula used to calculate the viscosity of methylcellulose. Why is the result expressed in centipoises instead of centistokes?

Chapter 28

ELECTROLYTIC METHODS

Quantitative analysis by means of electrolysis, or electroanalysis, as it is more generally called, is usually restricted to the determination of metals. This method of analysis is based upon the fact that an electrical current passed through a solution of the salt of a metal causes the deposition of the metal, usually in the elemental state, upon one of the electrodes. When deposition is complete the anode or cathode is removed, washed, dried, and weighed. The increase in weight over the tared electrode represents the weight of the substance deposited.

Theory. Those substances which conduct an electric current when dissolved in aqueous solution are termed *electrolytes.* Electrolytic determinations are based upon the fact that, when a direct current of electricity is passed through a solution of an electrolyte, some of the negatively charged ions are oxidized to the elemental state, and some of the positively charged ions are reduced to the elemental state. This decomposition of an electrolyte in solution is known as *electrolysis*, and when the decomposition of the electrolyte is carried out for purposes of analysis, the process is called *electroanalysis.* The conductor by which the current enters the solution of electrolyte is called the *anode*, and that by which it leaves the solution is known as the *cathode.* The anode and cathode are generally spoken of as positive and negative *electrodes*, respectively, or simply as electrodes. The source of current charges the positive electrode (anode) with positive electricity, and this anode attracts the negatively charged ions (*anions*), but it repels the positively charged ions (*cations*); the latter are attracted by the negatively charged cathode which repels the negatively charged anions, forcing them toward the anode. Since the positively charged ions gain an electron for each positive charge that they carry when they come in contact with the cathode, they are reduced; and since the negatively charged ions lose an electron for each negative charge that they carry when they come in contact with the anode, they are oxidized. The respective reductions and oxidations never take place in the mass of the solution of electrolyte but only at the electrodes, reduction occurring at the cathode and oxidation occurring at the anode. Simple metallic ions (cations), such as copper, mercury, silver, and zinc, travel to the cathode where they are reduced to the metallic state. The simple anions, such as chloride, bromide, or iodide, travel to the anode and are oxidized to the molecular condition, while solutions containing hydroxide, sulfate, or nitrate ions liberate oxygen at the anode. The halogen or oxygen liberated may escape from the solution or it may act directly or indirectly as an oxidizing agent.

The various anions are not all oxidized with the same ease, their relative order being sulfide, iodide, bromide, and chloride, where the first named is the most readily oxidized. Likewise, the different cations are not all reduced with the same ease, their relative order being auric, platinic, silver, mercurous, cupric, arsenous, bismuth, hydrogen, and lead, where the first named is most readily reduced. The electrolytic method of analysis is, therefore, a type of oxidation-reduction analysis, since the oxidation of one species of ion always takes place at the anode simultaneously with the reduction of another species of ion at the cathode.

Electrolytic methods of assay are not so simple as it might seem from the brief foregoing discussion, since numerous factors complicate conditions, *viz.*, resistance of the solution, polarization, overvoltage, reactions at the electrodes, the nature of the solutions used, the current density, the rate of stirring, the shape and size of the electrodes, and the temperature. Although numerous metals may be analyzed electrolytically and the method may be applied to a number of official substances, *viz.*, the salts of mercury, silver, copper, and zinc, there are no official electrolytic assays. This is true since other methods of analysis, in general, have certain advantages over electroanalysis procedures. Therefore, a discussion of the above-mentioned factors and the various types of apparatus and procedures is beyond the scope of the intent of this text; those who desire a more comprehensive knowledge of this subject should consult the previous edition of this book or the references listed below.

References

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APPENDIX

LOGARITHMS OF NUMBERS*

Natural numbers	0	1	2	3	4	5	6	7	8	9	Proportional parts								
											1	2	3	4	5	6	7	8	9
10	0000	0043	0086	0128	0170	0212	0253	0294	0334	0374	4	8	12	17	21	25	29	33	37
11	0414	0453	0492	0531	0569	0607	0645	0682	0719	0755	4	8	11	15	19	23	26	30	34
12	0792	0828	0864	0899	0934	0969	1004	1038	1072	1106	3	7	10	14	17	21	24	28	31
13	1139	1173	1206	1239	1271	1303	1335	1367	1399	1430	3	6	10	13	16	19	23	26	29
14	1461	1492	1523	1553	1584	1614	1644	1673	1703	1732	3	6	9	12	15	18	21	24	27
15	1761	1790	1818	1847	1875	1903	1931	1959	1987	2014	3	6	8	11	14	17	20	22	25
16	2041	2068	2095	2122	2148	2175	2201	2227	2253	2279	3	5	8	11	13	16	18	21	24
17	2304	2330	2355	2380	2405	2430	2455	2480	2504	2529	2	5	7	10	12	15	17	20	22
18	2553	2577	2601	2625	2648	2672	2695	2718	2742	2765	2	5	7	9	12	14	16	19	21
19	2788	2810	2833	2856	2878	2900	2923	2945	2967	2989	2	4	7	9	11	13	16	18	20
20	3010	3032	3054	3075	3096	3118	3139	3160	3181	3201	2	4	6	8	11	13	15	17	19
21	3222	3243	3263	3284	3304	3324	3345	3365	3385	3404	2	4	6	8	10	12	14	16	18
22	3424	3444	3464	3483	3502	3522	3541	3560	3579	3598	2	4	6	8	10	12	14	15	17
23	3617	3636	3655	3674	3692	3711	3729	3747	3766	3784	2	4	6	7	9	11	13	15	17
24	3802	3820	3838	3856	3874	3892	3909	3927	3945	3962	2	4	5	7	9	11	12	14	16
25	3979	3997	4014	4031	4048	4065	4082	4099	4116	4133	2	3	5	7	9	10	12	14	15
26	4150	4166	4183	4200	4216	4232	4249	4265	4281	4298	2	3	5	7	8	10	11	13	15
27	4314	4330	4346	4362	4378	4393	4409	4425	4440	4456	2	3	5	6	8	9	11	13	14
28	4472	4487	4502	4518	4533	4548	4564	4579	4594	4609	2	3	5	6	8	9	11	12	14
29	4624	4639	4654	4669	4683	4698	4713	4728	4742	4757	1	3	4	6	7	9	10	12	13
30	4771	4786	4800	4814	4829	4843	4857	4871	4886	4900	1	3	4	6	7	9	10	11	13
31	4914	4928	4942	4955	4969	4983	4997	5011	5024	5038	1	3	4	6	7	8	10	11	12
32	5051	5065	5079	5092	5105	5119	5132	5145	5159	5172	1	3	4	5	7	8	9	11	12
33	5185	5198	5211	5224	5237	5250	5263	5276	5289	5302	1	3	4	5	6	8	9	10	12
34	5315	5328	5340	5353	5366	5378	5391	5403	5416	5428	1	3	4	5	6	8	9	10	11
35	5441	5453	5465	5478	5490	5502	5514	5527	5539	5551	1	2	4	5	6	7	9	10	11
36	5563	5575	5587	5599	5611	5623	5635	5647	5658	5670	1	2	4	5	6	7	8	10	11
37	5682	5694	5705	5717	5729	5740	5752	5763	5775	5786	1	2	3	5	6	7	8	9	10
38	5798	5809	5821	5832	5843	5855	5866	5877	5888	5899	1	2	3	5	6	7	8	9	10
39	5911	5922	5933	5944	5955	5966	5977	5988	5999	6010	1	2	3	4	5	7	8	9	10
40	6021	6031	6042	6053	6064	6075	6085	6096	6107	6117	1	2	3	4	5	6	8	9	10
41	6128	6138	6149	6160	6170	6180	6191	6201	6212	6222	1	2	3	4	5	6	7	8	9
42	6232	6243	6253	6263	6274	6284	6294	6304	6314	6325	1	2	3	4	5	6	7	8	9
43	6335	6345	6355	6365	6375	6385	6395	6405	6415	6425	1	2	3	4	5	6	7	8	9
44	6435	6444	6454	6464	6474	6484	6493	6503	6513	6522	1	2	3	4	5	6	7	8	9
45	6532	6542	6551	6561	6571	6580	6590	6599	6609	6618	1	2	3	4	5	6	7	8	9
46	6628	6637	6646	6656	6665	6675	6684	6693	6702	6712	1	2	3	4	5	6	7	7	8
47	6721	6730	6739	6749	6758	6767	6776	6785	6794	6803	1	2	3	4	5	5	6	7	8
48	6812	6821	6830	6839	6848	6857	6866	6875	6884	6893	1	2	3	4	4	5	6	7	8
49	6902	6911	6920	6928	6937	6946	6955	6964	6972	6981	1	2	3	4	4	5	6	7	8
50	6990	6998	7007	7016	7024	7033	7042	7050	7059	7067	1	2	3	3	4	5	6	7	8
51	7076	7084	7093	7101	7110	7118	7126	7135	7143	7152	1	2	3	3	4	5	6	7	8
52	7160	7168	7177	7185	7193	7202	7210	7218	7226	7235	1	2	2	3	4	5	6	7	7
53	7243	7251	7259	7267	7275	7284	7292	7300	7308	7316	1	2	2	3	4	5	6	6	7
54	7324	7332	7340	7348	7356	7364	7372	7380	7388	7396	1	2	2	3	4	5	6	6	7

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* See page 22 for examples illustrating the use of logarithmic tables in calculations.

LOGARITHMS OF NUMBERS.* *(Continued)*

Natural numbers	0	1	2	3	4	5	6	7	8	9	Proportional parts								
											1	2	3	4	5	6	7	8	9
55	7404	7412	7419	7427	7435	7443	7451	7459	7466	7474	1	2	2	3	4	5	5	6	7
56	7482	7490	7497	7505	7513	7520	7528	7536	7543	7551	1	2	2	3	4	5	5	6	7
57	7559	7566	7574	7582	7589	7597	9604	7612	7619	7627	1	2	2	3	4	5	5	6	7
58	7634	7642	7649	7657	7664	7672	7679	7686	7694	7701	1	1	2	3	4	4	5	6	7
59	7709	7716	7723	7731	7738	7745	7752	7760	7767	7774	1	1	2	3	4	4	5	6	7
60	7782	7789	7796	7803	7810	7818	7825	7832	7839	7846	1	1	2	3	4	4	5	6	6
61	7853	7860	7868	7875	7882	7889	7896	7903	7910	7917	1	1	2	3	4	4	5	6	6
62	7924	7931	7938	7945	7952	7959	7966	7973	7980	7987	1	1	2	3	3	4	5	6	6
63	7993	8000	8007	8014	8021	8028	8035	8041	8048	8055	1	1	2	3	3	4	5	5	6
64	8062	8069	8075	8082	8089	8096	8102	8109	8116	8122	1	1	2	3	3	4	5	5	6
65	8129	8136	8142	8149	8156	8162	8169	8176	8182	8189	1	1	2	3	3	4	5	5	6
66	8195	8202	8209	8215	8222	8228	8235	8241	8248	8254	1	1	2	3	3	4	5	5	6
67	8261	8267	8274	8280	8287	8293	8299	8206	8312	8319	1	1	2	3	3	4	5	5	6
68	8325	8331	8338	8344	8351	8357	8363	8370	8376	8382	1	1	2	3	3	4	4	5	6
69	8388	8395	8401	8407	8414	8420	8426	8432	8439	8445	1	1	2	2	3	4	4	5	6
70	8451	8457	8463	8470	8476	8482	8488	8494	8500	8506	1	1	2	2	3	4	4	5	6
71	8513	8519	8525	8531	8537	8543	8549	8555	8561	8567	1	1	2	2	3	4	4	5	5
72	8573	8579	8585	8591	8597	8603	8609	8615	8621	8627	1	1	2	2	3	4	4	5	5
73	8633	8639	8645	8651	8657	8663	8669	8675	8681	8686	1	1	2	2	3	4	4	5	5
74	8692	8698	8704	8710	8716	8722	8727	8733	8739	8745	1	1	2	2	3	4	4	5	5
75	8751	8756	8762	8768	8774	8779	8785	8791	8797	8802	1	1	2	2	3	3	4	5	5
76	8808	8814	8820	8825	8831	8837	8842	8848	8854	8859	1	1	2	2	3	3	4	5	5
77	8865	8871	8876	8882	8887	8893	8899	8904	8910	8915	1	1	2	2	3	3	4	4	5
78	8921	8927	8932	8938	8943	8949	8954	8960	8965	8971	1	1	2	2	3	3	4	4	5
79	8976	8982	8987	8993	8998	9004	9009	9015	9020	9026	1	1	2	2	3	3	4	4	5
80	9031	9036	9042	9047	9053	9058	9063	9069	9074	9079	1	1	2	2	3	3	4	4	5
81	9085	9090	9096	9101	9106	9112	9117	9122	9128	9133	1	1	2	2	3	3	4	4	5
82	9138	9143	9149	9154	9159	9165	9170	9175	9180	9186	1	1	2	2	3	3	4	4	5
83	9191	9196	9201	9206	9212	9217	9222	9227	9232	9238	1	1	2	2	3	3	4	4	5
84	9243	9248	9253	9258	9263	9269	9274	9279	9284	9289	1	1	2	2	3	3	4	4	5
85	9294	9299	9304	9309	9315	9320	9325	9330	9335	9340	1	1	2	2	3	3	4	4	5
86	9345	9350	9355	9360	9365	9370	9375	9380	9385	9390	1	1	2	2	3	3	4	4	5
87	9395	9400	9405	9410	9415	9420	9425	9430	9435	9440	0	1	1	2	2	3	3	4	4
88	9445	9450	9455	9460	9465	9469	9474	9479	9484	9489	0	1	1	2	2	3	3	4	4
89	9494	9499	9504	9509	9513	9518	9523	9528	9533	9538	0	1	1	2	2	3	3	4	4
90	9542	9547	9552	9557	9562	9566	9571	9576	9581	9586	0	1	1	2	2	3	3	4	4
91	9590	9595	9600	9606	9609	9614	9619	9624	9628	9633	0	1	1	2	2	3	3	4	4
92	9638	9643	9647	9652	9657	9661	9666	9671	9675	9680	0	1	1	2	2	3	3	4	4
93	9685	9689	9694	9699	9703	9708	9713	9717	9722	9727	0	1	1	2	2	3	3	4	4
94	9731	9736	9741	9745	9750	9754	9759	9763	9768	9773	0	1	1	2	2	3	3	4	4
95	9777	9782	9786	9791	9795	9800	9805	9809	9814	9818	0	1	1	2	2	3	3	4	4
96	9823	9827	9832	9836	9841	9845	9850	9854	9859	9863	0	1	1	2	2	3	3	4	4
97	9868	9872	9877	9818	9886	9890	9894	9899	9903	9908	0	1	1	2	2	3	3	4	4
98	9912	9917	9921	9926	9930	9934	9939	9943	9948	9952	0	1	1	2	2	3	3	4	4
99	9956	9961	9965	9969	9974	9978	9983	9987	9991	9996	0	1	1	2	2	3	3	3	4

ANTILOGARITHMS

Logarithms	0	1	2	3	4	5	6	7	8	9	Proportional parts								
											1	2	3	4	5	6	7	8	9
0.00	1000	1002	1005	1007	1009	1012	1014	1016	1019	1021	0	0	1	1	1	1	2	2	2
0.01	1023	1026	1028	1030	1033	1035	1038	1040	1042	1045	0	0	1	1	1	1	2	2	2
0.02	1047	1050	1052	1054	1057	1059	1062	1064	1067	1069	0	0	1	1	1	1	2	2	2
0.03	1072	1074	1076	1079	1081	1084	1086	1089	1091	1094	0	0	1	1	1	1	2	2	2
0.04	1096	1099	1102	1104	1107	1109	1112	1114	1117	1119	0	1	1	1	1	2	2	2	2
0.05	1122	1125	1127	1130	1132	1135	1138	1140	1143	1146	0	1	1	1	1	2	2	2	2
0.06	1148	1151	1153	1156	1159	1161	1164	1167	1169	1172	0	1	1	1	1	2	2	2	2
0.07	1175	1178	1180	1183	1186	1189	1191	1194	1197	1199	0	1	1	1	1	2	2	2	2
0.08	1202	1205	1208	1211	1213	1216	1219	1222	1225	1227	0	1	1	1	1	2	2	2	3
0.09	1230	1233	1236	1239	1242	1245	1247	1250	1253	1256	0	1	1	1	1	2	2	2	3
0.10	1259	1262	1265	1268	1271	1274	1276	1279	1282	1285	0	1	1	1	1	2	2	2	3
0.11	1288	1291	1294	1297	1300	1303	1306	1309	1312	1315	0	1	1	1	2	2	2	2	3
0.12	1318	1321	1324	1327	1330	1334	1337	1340	1343	1346	0	1	1	1	2	2	2	2	3
0.13	1349	1352	1355	1358	1361	1365	1368	1371	1374	1377	0	1	1	1	2	2	2	3	3
0.14	1380	1384	1387	1390	1393	1396	1400	1403	1406	1409	0	1	1	1	2	2	2	3	3
0.15	1413	1416	1419	1422	1426	1429	1432	1435	1439	1442	0	1	1	1	2	2	2	3	3
0.16	1445	1449	1452	1455	1459	1462	1466	1469	1472	1476	0	1	1	1	2	2	2	3	3
0.17	1479	1483	1486	1489	1493	1496	1500	1503	1507	1510	0	1	1	1	2	2	2	3	3
0.18	1514	1517	1521	1524	1528	1531	1535	1538	1542	1545	0	1	1	1	2	2	2	3	3
0.19	1549	1552	1556	1560	1563	1567	1570	1574	1578	1581	0	1	1	1	2	2	3	3	3
0.20	1585	1589	1592	1596	1600	1603	1607	1611	1614	1618	0	1	1	1	2	2	3	3	3
0.21	1622	1626	1629	1633	1637	1641	1644	1648	1652	1656	0	1	1	2	2	2	3	3	3
0.22	1660	1663	1667	1671	1675	1679	1683	1687	1690	1694	0	1	1	2	2	2	3	3	3
0.23	1698	1702	1706	1710	1714	1718	1722	1726	1730	1734	0	1	1	2	2	2	3	3	4
0.24	1738	1742	1746	1750	1754	1758	1762	1766	1770	1774	0	1	1	2	2	2	3	3	4
0.25	1778	1782	1786	1791	1795	1799	1803	1807	1811	1816	0	1	1	2	2	2	3	3	4
0.26	1820	1824	1828	1832	1837	1841	1845	1849	1854	1858	0	1	1	2	2	3	3	3	4
0.27	1862	1866	1871	1875	1879	1884	1888	1892	1897	1901	0	1	1	2	2	3	3	3	4
0.28	1905	1910	1914	1919	1923	1928	1932	1936	1941	1945	0	1	1	2	2	3	3	4	4
0.29	1950	1954	1959	1963	1968	1972	1977	1982	1986	1991	0	1	1	2	2	3	3	4	4
0.30	1995	2000	2004	2009	2014	2018	2023	2028	2032	2037	0	1	1	2	2	3	3	4	4
0.31	2042	2046	2051	2056	2061	2065	2070	2075	2080	2084	0	1	1	2	2	3	3	4	4
0.32	~~2089~~	2094	2099	2104	2109	2113	2118	2123	2128	2133	0	1	1	2	2	3	3	4	4
0.33	2138	2143	2148	2153	2158	2163	2168	2173	2178	2183	0	1	1	2	2	3	3	4	4
0.34	2188	2193	2198	2203	2208	2213	2218	2223	2228	2234	1	1	2	2	3	3	4	4	5
0.35	2239	2244	2249	2254	2259	2265	2270	2275	2280	2286	1	1	2	2	3	3	4	4	5
0.36	2291	2296	2301	2307	2312	2317	2323	2328	2333	2339	1	1	2	2	3	3	4	4	5
0.37	2344	2350	2355	2360	2366	2371	2377	2382	2388	2393	1	1	2	2	3	3	4	4	5
0.38	2399	2404	2410	2415	2421	2427	2432	2438	2443	2449	1	1	2	2	3	3	4	4	5
0.39	2455	2460	2466	2472	2477	2483	2489	2495	2500	2506	1	1	2	2	3	3	4	5	5
0.40	2512	2518	2523	2529	2535	2541	2547	2553	2559	2564	1	1	2	2	3	4	4	5	5
0.41	2570	2576	2582	2588	2594	2600	2606	2612	2618	2624	1	1	2	2	3	4	4	5	5
0.42	2630	2636	2642	2649	2655	2661	2667	2673	2679	2685	1	1	2	2	3	4	4	5	6
0.43	2692	2698	2704	2710	2716	2723	2729	2735	2742	2748	1	1	2	3	3	4	4	5	6
0.44	2754	2761	2767	2773	2780	2786	2793	2799	2805	2812	1	1	2	3	3	4	4	5	6
0.45	2818	2825	2831	2838	2844	2851	2858	2864	2871	2877	1	1	2	3	3	4	5	5	6
0.46	2884	2891	2897	2904	2911	2917	2924	2931	2938	2944	1	1	2	3	3	4	5	5	6
0.47	2951	2958	2965	2972	2979	2985	2992	2999	3006	3013	1	1	2	3	3	4	5	5	6
0.48	3020	3027	3034	3041	3048	3055	3062	3069	3076	3083	1	1	2	3	4	4	5	6	6
0.49	3090	3097	3105	3112	3119	3126	3133	3141	3148	3155	1	1	2	3	4	4	5	6	6

ANTILOGARITHMS. *(Continued)*

Logarithms	0	1	2	3	4	5	6	7	8	9	Proportional parts								
											1	2	3	4	5	6	7	8	9
0.50	3162	3170	3177	3184	3192	3199	3206	3214	3221	3228	1	1	2	3	4	4	5	6	7
0.51	3236	3243	3251	3258	3266	3273	3281	3289	3296	3304	1	2	2	3	4	5	5	6	7
0.52	3311	3319	3327	3334	3342	3350	3357	3365	3373	3381	1	2	2	3	4	5	5	6	7
0.53	3388	3396	3404	3412	3420	3428	3436	3443	3451	3459	1	2	2	3	4	5	6	6	7
0.54	3467	3475	3483	3491	3499	3508	3516	3524	3532	3540	1	2	2	3	4	5	6	6	7
0.55	3548	3556	3565	3573	3581	3589	3597	3606	3614	3622	1	2	2	3	4	5	6	7	7
0.56	3631	3639	3648	3656	3664	3673	3681	3690	3698	3707	1	2	3	3	4	5	6	7	8
0.57	3715	3724	3733	3741	3750	3758	3767	3776	3784	3793	1	2	3	3	4	5	6	7	8
0.58	3802	3811	3819	3828	3837	3846	3855	3864	3873	3882	1	2	3	4	4	5	6	7	8
0.59	3890	3899	3908	3917	3926	3936	3945	3954	3963	3972	1	2	3	4	5	5	6	7	8
0.60	3981	3990	3999	4009	4018	4027	4036	4046	4055	4064	1	2	3	4	5	6	6	7	8
0.61	4074	4083	4093	4102	4111	4121	4130	4140	4150	4159	1	2	3	4	5	6	7	8	9
0.62	4169	4178	4188	4198	4207	4217	4227	4236	4246	4256	1	2	3	4	5	6	7	8	9
0.63	4266	4276	4285	4295	4305	4315	4325	4335	4345	4355	1	2	3	4	5	6	7	8	9
0.64	4365	4375	4385	4395	4406	4416	4426	4436	4446	4457	1	2	3	4	5	6	7	8	9
0.65	4467	4477	4487	4498	4508	4519	4529	4539	4550	4560	1	2	3	4	5	6	7	8	9
0.66	4571	4581	4592	4603	4613	4624	4634	4645	4656	4667	1	2	3	4	5	6	7	9	10
0.67	4677	4688	4699	4710	4721	4732	4742	4753	4764	4775	1	2	3	4	5	7	8	9	10
0.68	4786	4797	4808	4819	4831	4842	4853	4864	4875	4887	1	2	3	4	6	7	8	9	10
0.69	4898	4909	4920	4932	4943	4955	4966	4977	4989	5000	1	2	3	5	6	7	8	9	10
0.70	5012	5023	5035	5047	5058	5070	5082	5093	5105	5117	1	2	4	5	6	7	8	9	11
0.71	5129	5140	5152	5164	5176	5183	5200	5212	5224	5236	1	2	4	5	6	7	8	10	11
0.72	5248	5260	5272	5284	5297	5309	5321	5333	5346	5358	1	2	4	5	6	7	9	10	11
0.73	5370	5383	5395	5408	5420	5433	5445	5458	5470	5483	1	3	4	5	6	8	9	10	11
0.74	5495	5508	5521	5534	5546	5559	5572	5585	5598	5610	1	3	4	5	6	8	9	10	12
0.75	5623	5636	5649	5662	5675	5689	5702	5715	5728	5741	1	3	4	5	7	8	9	10	12
0.76	5754	5768	5781	5794	5808	5821	5834	5848	5861	5875	1	3	4	5	7	8	9	11	12
0.77	5888	5902	5916	5929	5943	5957	5970	5984	5998	6012	1	3	4	5	7	8	10	11	12
0.78	6026	6039	6053	6067	6081	6095	6109	6124	6138	6152	1	3	4	6	7	8	10	11	13
0.79	6166	6180	6194	6209	6223	6237	6252	6266	6281	6295	1	3	4	6	7	9	10	11	13
0.80	6310	6324	6339	6353	6368	6383	6397	6412	6427	6442	1	3	4	6	7	9	10	12	13
0.81	6457	6471	6486	6501	6516	6531	6546	6561	6577	6592	2	3	5	6	8	9	11	12	14
0.82	6607	6622	6637	6653	6668	6683	6699	6714	6730	6745	2	3	5	6	8	9	11	12	14
0.83	6761	6776	6792	6808	6823	6839	6855	6871	6887	6902	2	3	5	6	8	9	11	13	14
0.84	6918	6934	6950	6966	6982	6998	7015	7031	7047	7063	2	3	5	6	8	10	11	13	15
0.85	7079	7096	7112	7129	7145	7161	7178	7194	7211	7228	2	3	5	7	8	10	12	13	15
0.86	7244	7261	7278	7295	7311	7328	7345	7362	7379	7396	2	3	5	7	8	10	12	13	15
0.87	7413	7430	7447	7464	7482	7499	7516	7534	7551	7568	2	3	5	7	9	10	12	14	16
0.88	7586	7603	7621	7638	7656	7674	7691	7709	7727	7745	2	4	5	7	9	11	12	14	16
0.89	7762	7780	7798	7816	7834	7852	7870	7889	7907	7925	2	4	5	7	9	11	13	14	16
0.90	7943	7962	7980	7998	8017	8035	8054	8072	8091	8110	2	4	6	7	9	11	13	15	17
0.91	8128	8147	8166	8185	8204	8222	8241	8260	8279	8299	2	4	6	8	9	11	13	15	17
0.92	8318	8337	8356	8375	8395	8414	8433	8453	8472	8492	2	4	6	8	10	12	14	15	17
0.93	8511	8531	8551	8570	8590	8610	8630	8650	8670	8690	2	4	6	8	10	12	14	16	18
0.94	8710	8730	8750	8770	8790	8810	8831	8851	8872	8892	2	4	6	8	10	12	14	16	18
0.95	8913	8933	8954	8974	8995	9016	9036	9057	9078	9099	2	4	6	8	10	12	15	17	19
0.96	9120	9141	9162	9183	9204	9226	9247	9268	9290	9311	2	4	6	8	11	13	15	17	19
0.97	9333	9354	9376	9397	9419	9441	9462	9484	9506	9528	2	4	7	9	11	13	15	17	20
0.98	9550	9572	9594	9616	9638	9661	9683	9705	9727	9750	2	4	7	9	11	13	16	18	20
0.99	9772	9795	9817	9840	9863	9886	9908	9931	9954	9977	2	5	7	9	11	14	16	18	20

LIST OF VISUAL AIDS

The visual materials listed below and on the following pages can be used to supplement the material in this book. It is recommended, however, that each film be reviewed before using in order to determine its suitability for a particular group.

Both motion pictures and filmstrips are included in this list of visual materials, and the character of each one is indicated by the self-explanatory abbreviations "MP" and "FS." Immediately following this identification is the name of the producer; and if the distributor is different from the producer, the name of the distributor follows the name of the producer. Abbreviations are used for names of producers and distributors, and these abbreviations are identified in the list of sources at the end of the bibliography. In most instances, the films can be borrowed or rented from local or state 16mm film libraries. (A nationwide list of these local sources is given in "A Directory of 2002 16mm Film Libraries," available for thirty-five cents from the Superintendent of Documents, Washington 25, D.C.) Unless otherwise indicated, the motion pictures are 16mm black and white sound films and the filmstrips are 35mm black and white silent. The running time (min) is listed with each film and the number of frames (fr) for each filmstrip.

This bibliography is suggestive only, and film users should examine the latest annual edition and quarterly supplements of "Educational Film Guide," a catalogue of some 10,000 films published by the H. W. Wilson Company, New York, N.Y. The "Guide," a standard reference book, is available in most college and public libraries.

CHEMISTRY

Analytical Balance (MP; Minn U; 15min). Demonstrates the operation and uses of an analytical balance.

Basic Principles of the Analytical Balance (MP; USPHS/UWF 19min). Explains the uses of the analytical balance and shows ho to find the zero point of the balance, find its sensitivity, calibrat the weights, weigh an object, and weigh out a predetermined amoun of material. Filmstrip (100 *fr with disc;* 13*min*), same title, coverin same material also available.

Catalysis (MP; EBF; 11min). Explains phenomena associate with catalysis, including concentration by absorption, activation b absorption, intermediate compounds in a homogeneous mixture chain reactions, and negative catalysts or inhibitors.

Chemistry (FS series; McGraw). A series of nine filmstrips portraying and explaining fundamental concepts of chemistry, as indicated in the following titles of the individual filmstrips:

The Kinetic Molecular Theory (59 fr)
The Atomic Theory (45 fr)
The Chemical Formula (55 fr)
Equations (35 fr)
The Structure of the Atom (49 fr)
Ionization (42 fr)
Acid and Basic Solutions (43 fr)
Electrolysis (42 fr)
The Periodic Table (50 fr)

Colloids (MP; EBF; 11min). Shows colloidal suspensions such as liquid in gas, gas in liquid, solid in gas, liquid in liquid; Brownian movement; and the Cottrell process of smoke precipitation.

Colloids and their Behavior (MP; Rolab; 30min si). Covers classification of types, preparation, colloids in nature, various physicochemical reactions, and coagulation.

Electrochemistry (MP; EBF; 11min). Illustrates through various examples the principles of electrochemistry.

Electrolysis (MP; McGraw; 5min). Explains the conduction of electricity through a liquid.

The Electroscope (MP; McGraw; 5min). Deals with the detection of electric charges.

Flue Gas Analysis (*Orsat Apparatus*) (MP; USOE/UWF; 19min).

How to set up the Orsat gas analyzer; collect an air-free sample of flue gas; absorb and measure the amounts of carbon dioxide, oxygen, and carbon monoxide in the sample; and calculate the amount of nitrogen. Filmstrip (43 *fr*), same title, covering same material also available.

Gravimetric Quantitative Analysis (MP; PSC; 11min si). Explains the use of the analytical balance and the steps involved in precipitation and weighing.

Oxidation and Reduction (MP; EBF; 11min). Illustrates the simultaneous processes of oxidation and reduction and explains the principles underlying the processes.

Pictorial Chemistry (FS series; SVE). A series of four filmstrips, approximately 50 frames each, explaining chemical properties through the use of photographs, diagrams, cartoons, and test questions. Titles of the individual filmstrips are:

Acids, Bases, and Salts
Halogens—A Typical Chemical Family
Ionization and Electrolysis
Solutions, Colloids, and Suspension

PHARMACOLOGY

Globulin Modified Antitoxin (MP; Lederle; 43min si). Shows the production of diphtheria antitoxin, testing, purification, packaging.

Immunization Against Infectious Diseases (MP; Lederle; 44min si color). Shows the active and passive immunization procedures against infectious diseases.

Industry for Health (MP; Upjohn/MTP; 30min). Laboratory procedures in the development of drugs; testing for medicinally active substances and impurities; extraction and adjustment to standards; and preparation in pill, capsule, powder, liquid, and other forms for administration.

Miracle Drugs of the Century (MP; Nat Pic; 15min). Characteristics, accomplishments, and limitations of the sulfonamides.

New Frontiers of Medicine (MP; MOT/McGraw; 17min). Reviews recent (1948) discoveries in medicine including the sulfa drugs, penicillin, and streptomycin.

Penicillin in Medical Practice (MP; BIS; 33min). Nature and properties of penicillin, storage, methods of use, examples of use in surgery and medicine.

The Physiologic Basis for the Action of ACTH in Human Beings (MP; Armour; 37min color). Explains the physiological basis for the clinical actions of *ACTH.*

Squibb's Rx (MP; Squibb; 20min). Explains the development and manufacture of drugs, and emphasizes the need for continuous research.

Streptomycin (MP; USA/UWF; 10min). Reviews the events and work that led to the discovery of the drug streptomycin by Dr. Waxman at Rutgers University.

Sulfonamide Therapy (MP; Lederle; 45min si color). Gives good historical background; shows developments leading to introduction of sulfadiazine.

SOURCES OF FILMS LISTED ABOVE

Armour—Armour Laboratories, 1425 West 42d St., Chicago 9, Ill.
BIS—British Information Services, 30 Rockefeller Plaza, New York 20, N.Y.
EBF—Encyclopaedia Britannica Films, 1150 Wilmette Ave., Wilmette, Ill.
Lederle—Lederle Laboratories, 30 Rockefeller Plaza, New York 20, N.Y.
McGraw—McGraw-Hill Book Co., Text-Film Dept., 330 W. 42d St., New York 36, N.Y.
Minn U—University of Minnesota, Minneapolis, Minn.
MOT—March of Time Forum Films, 369 Lexington Ave., New York 17, N.Y.
MTP—Modern Talking Picture Service, 9 Rockefeller Plaza, New York 20, N.Y.
Nat Pic—National Motion Picture Co., Mooresville, Ind.
PSC—Pennsylvania State College, Audio-Visual Aids Library, State College, Pa.
Rolab—Rolab Laboratories, Sandy Hook, Conn.
Squibb—E. R. Squibb & Sons, 745 Fifth Ave., New York 22, N.Y.

SVE—Society for Visual Education, Inc., 1345 W. Diversey Pkwy., Chicago 14, Ill.
USA—U.S. Department of the Army, Washington 25, D.C.
USOE—U.S. Office of Education, Washington 25, D.C.
USPHS—U.S. Public Health Service, Washington 25, D.C.
Upjohn—Upjohn Co., Kalamazoo, Mich. (Film distributed by MTP).
UWF—United World Films, Inc., 1445 Park Ave., New York 29, N.Y.

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